On inner warfare, the host and the adversary: public health genomics of chlamydia trachomatis and human papillomavirus

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On inner warfare, the host and the adversary: Public Health Genomics of *Chlamydia trachomatis* and human papillomavirus

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There are known knowns. These are things we know that we know.
There are known unknowns. That is to say, there are things that we know we don't know.
But there are also unknown unknowns.
There are things we don't know we don't know.
Donald Rumsfeld

Contents

INTRODUCTION

The Initial Mystery that attends any journey is:
how did the traveller reach his starting point in the first place?
Louise Bogan*, Journey Around My Room*

General introduction

The second half of the 20th century has witnessed unprecedented drops in infection mortality and morbidity rates due to the impact of several milestone medical achievements, such as vaccines and antimicrobials (WHO, 2010), as well as hygienic advancements and the development of infrastructure. This, along with changes in lifestyle, has inevitably led to a new epidemiological transition in the developed world, bringing several chronic diseases to the forefront of disease mortality and morbidity. A large number of developing countries, however, continued dealing with a high burden of infectious diseases. The emergence of new infectious diseases, such as HIV, but also the more recent threats - SARS, MERS, multiresistant strains of highly pathogenic bacteria - have yet again brought basic scientists' attention to the research of infections. Additionally, the impact of these outbreaks is a global phenomenon that threatens both the developing and the developed world.

To this day, despite these landmark accomplishments in the field of preventive medicine and therapeutics, infectious diseases rank as high as second on the list of global causes of death (WHO, 2012). Moreover, these conditions also represent one of the leading causes of morbidity (WHO, 2009). HIV/AIDS still remains one of the main communicable global causes of death, while several other sexually transmitted infections (STIs) also contribute to overall morbidity, as well as incurred psychological burden and economic costs (Weinstock *et al.,* 2004). Uncleared infections by STIs such as *Chlamydia trachomatis* and human papillomavirus (HPV) are crucial contributors to morbidity in women and humans in general. Decades of screening have led to a relative drop in prevalence of diseases for which these uncleared infections play a role in, however there are pronounced differences in the effect of screening programmes when compared between different groups.

The stumbling stones of current prevention strategies have their roots in 'one-size-fits-all' paradigm that by its definition neglects innate differences in both the host and the pathogen (Malik, 2013). Public Health Genomics brings a novel approach that can enable a more successful prevention, screening, diagnostics and treatment of infectious diseases. This thesis explores the opportunities for this approach that may contribute to tackling infections with *C. trachomatis* and HPV, as well as their related sequelae, The cornerstone of the Public Health Genomics approach in this respect represents inter-individual variation in susceptibility to and the course of infections and development of these sequelae. This variation is confined to the genes whose products are directly or circumstantially involved in the regulation of the immune response. Furthermore, environmental changes and socio-cultural elements intersect with these immunogenetic factors, creating a unique setting which will ultimately determine the character of each

individual's health outcome. By compiling and analysing empirical knowledge of these factors and proposing pathways for translating this knowledge into successful clinical applications, management of these infections and their complications may be advanced.

Contents of this chapter

1. *Chlamydia trachomatis*

1.1 Epidemiology and control programmes

*O*ver 100 million people globally are infected with *Chlamydia trachomatis* each year (WHO, 2012). Approximately 60.000 of these cases are being annually diagnosed in the Netherlands (Trienekens *et al.,* 2012). In developed countries, the highest prevalence is estimated to be among young women, age 15-24. In men the prevalence peaks at a later age than in girls (Satterwhite *et al.,* 2013; Haar *et al.,* 2013). Certain subgroups have however been linked to a higher prevalence of *C. trachomatis.* In Dutch men who have sex with men (MSM) it has been estimated to be at roughly 10% (Trienekens *et al.,* 2012). Prevalence rate was also found to be several times higher in certain minority groups. In Surinamese and Antillean population in the Netherlands, the observed prevalence was 8-12% (Gotz *et al.,* 2005; van Bergen *et al.,* 2005; van Bergen *et al.,* 2006). In most ethnic groups (with the exception of Turkish/Moroccan) the observed prevalence has been higher in women than men (van Bergen, 2005). Age of adolescence, having a new sex partner, multiple sex partners, lower partner referral, denial of condom use, and poor healthcare-seeking behaviour have been highlighted as the most important risk factors for *C. trachomatis* (Verhoeven *et al.,* 2003; Haar *et al.,* 2013).

Many high-income countries STI have instated control and management programmes, and *C. trachomatis* is usually a part of these programmes (Low *et al.,* 2012). In the Netherlands, people have access to STI screening via their general practitioner and the municipal health service (GGD STI centres) amongst others. Certain risk groups, including MSM and sex workers, have access to testing and treatment free of charge. Guidelines currently in place are based on sexual history. Once a case of infection is detected, active case management and contact tracing usually takes place. The Dutch system of STI management is quite developed, compared to other European countries (Low *et al.,* 2012).

1.2 Detection and treatment of Chlamydia trachomatis

Since the earliest detection approaches entered clinical practice in the early 20th century, the field has witnessed several generations of techniques enabling the detection of *C. trachomatis* (Warren *et al.,* 1993). The earliest laboratory tests, at the time referred to as the "gold standard", were the cell culture for *Chlamydiae* and antigen detection using immunofluorescence or enzyme immunoassay (Bauwens *et al.,* 1993; Warren *et al.,* 1993). Landmark developments in applied biochemistry, mainly the invention of polymerase chain reaction (PCR), have also revolutionised detection of pathogenic microorganisms

such as *Chlamydiae* (Loeffelholz *et al.,* 1992; Jaschek *et al.,* 1993). Main detection techniques for *C. trachomatis* currently used are PCR-based nucleic acid amplification tests (NAAT). *Chlamydia* antibody testing (CAT) is also used, however it is standardly done only as part of the diagnostic approach for *C. trachomatis*-related late complications, such as subfertility (Land *et al.,* 1998). These tests differ in their accuracy, with NAAT exhibiting higher sensitivity and specificity (Goessens, 1997).

NAATs are based on specific DNA or RNA amplification of *C. trachomatis*, making these tests highly specific and sensitive, and less labour intensive than the other tests (Doing *et al.,* 1999; Jespersen *et al.,* 2005). These diagnostic tests generate quick results and are currently the golden standard for *C. trachomatis* detection. The tests are widely used and due to technological advances they became less expensive. The advancement of NAAT testing and relatively successful standard treatment (single dose of azithromycin, or doxycycline as an alternative) has turned *C. trachomatis* into a manageable infection (Lau *et al.,* 2002; Brunham *et al.,* 2008). The rationale behind establishing control programmes was to improve women's reproductive health by shortening the infection period in patients and thus reducing the chance of transmission onto sexual partners (Brunham *et al.,* 2005). The alternative to a 1g single azithromycin regimen is therapy with doxycycline, 100mg for seven days. The effectiveness of this medication has been proven to be good (Lau, 2002).

1.3 Clinical manifestations

The majority of *C. trachomatis* infections remain asymptomatic, an average 70% of women and up to 50% of men (Zimmerman, 1990). The clearance rate in asymptomatic women within one year of diagnosing the infection appears to be 45-50% (Morré *et al.,* 2002; Molano *et al.,* 2005; Geisler *et al.,* 2008). There are however considerable discrepancies in clearance rates between different studies (Geisler, 2010). This is in part due to the difficulty of identifying when the infection initially occurred, or a failure to determine whether it is the case of a persistent or a new infection. Abnormal discharge, dysuria, and post-coital bleeding are among the most typical accompanying symptoms in women (Paavonen & Wolner-Hanssen, 1989). *C. trachomatis* infection can ascend into the upper genital tract. Most common sequelae of the infection advancing are pelvic inflammatory disease (PID), ectopic pregnancy, and tubal infertility (Hafner & Pelzer, 2011). The symptoms reported by men are generally dysuria and urethral discharge, and epididymitis and proctitis being the late complications in un-treated or un-cleared infection (Peipert, 2003; Cunningham & Beagley, 2008). There are pronounced differences between individuals in respect to clinical manifestations, and they can party be explained by the presence of different *C. trachomatis* genovars and serovars, whereas the

environment can play a role as well (van Duynhoven *et al.,* 1998; Morré *et al.,* 2000; Gomes *et al.,* 2006). The evidence for the role of these factors provides only a limited account for the differences in clinical outcomes of infected patients.

1.4 Pathogenesis and immune response

The sequenced chlamydial genome consists of a 1,042,519–base pair chromosome (58.7% A+T) and a 7493–base pair plasmid. Eight hundred and ninety four protein-coding genes have so far been identified (Stephens *et al.,* 1998). *C. trachomatis* is an obligate intracellular bacterial species, and it is entirely dependent on the host for nutrients and overall survival (Zomorodiour & Andersson, 1999). Attachment to and successful invasion of columnar epithelial cells by the infectious stadia of *C. trachomatis,* elementary bodies (EB), ideally sets off an immune response (Mascellino *et al.,* 2011). Upon establishing itself successfully within the host cell, EBs are converted to metabolically active reticulate bodies (RB), which replicate by binary fission inside inclusion bodies in the host cell (AbdelRahman *et al.,* 2005). The pathogen is capable of evading the fusion with lysosomes, which could signal its presence to the immune cells and trigger an inflammatory response, and that can ultimately result in its early eradication. RBs convert into EBs within 24-48h, which are released by controlled exocytosis and start a new cycle by infecting other epithelial cells (figure 1).

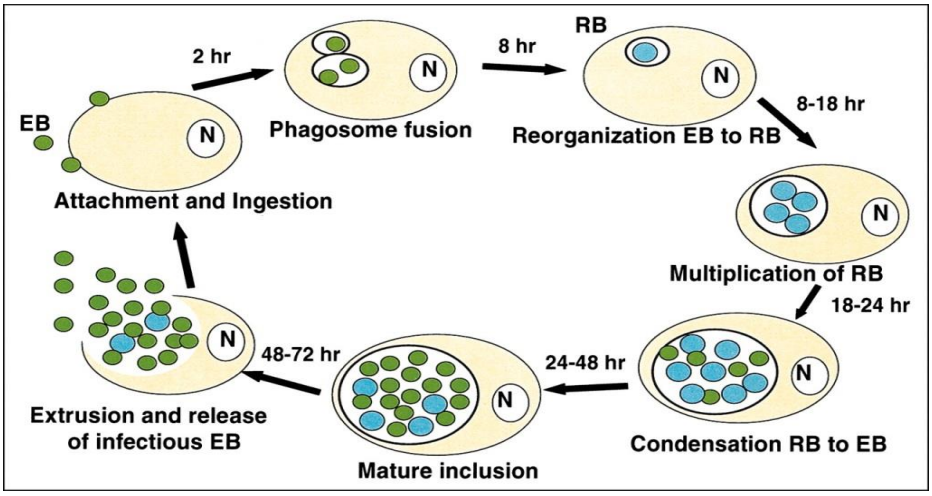

Figure 1. *Life cycle of C. trachomatis (adapted from Hammerschlag et al., 2004).*

Tissue residing macrophages and other antigen-presenting cells (APC), such as dendritic cells (DCs), engulf *C. trachomatis* cells and digest them within their phagolysosomes (Yong *et al.,* 1986). Peptide fragments are then associated with MHC class II molecules and presented at the surface where they can be detected by T-cells (Karunakaran *et al.,* 2008). Upon successful detection, the secretion of proinflammaroty cytokines drives the proliferation and differentiation of CD4+ T-helper cells into T_h1 subset, the main effector cells in host response to *C. trachomatis* infection. T_h1 cells secrete other cytokines (namely IFN-γ, IL-2 and IL-12) that eventually augment local inflammation. These cytokines, IFN-γ in particular, further activate the macrophages and other phagocytes, as well as expression of MHC class I and II molecules on epithelial cells (Darville & Hiltke, 2010). IFN-γ also activates the production of enzymes that metabolise tryptophan, thereby depleting the pathogen from this nutrient. *C. trachomatis* has, however, evolved an evasion mechanism – it is capable of producing its own tryptophan synthase (Nelson *et al.,* 2005).
As one of the outcomes of NFκB-induced immune response, B-cells start to secrete antibodies (immunoglobulin G (IgG)) and thereby aid the macrophages in opsonising *Chlamydia* particles (Wahl *et al.,* 2006). Research has shown that antibodies against *C. trachomatis* do not provide life-long protection against re-infection (den Hartog *et al.,* 2006). An ongoing immune response is down-regulated by the actions of an anti-inflammatory cytokine, IL-10, produced by T_h2-cells and regulatory T cells (T_{reg}), whose actions involve inhibiting NFκB (Moore, 2001). Upon establishing itself within intracellular inclusions, *C. trachomatis* evades recognition by supressing MHC-I expression of the host cell. Cells of certain human tissues, however, express intracellular pathogen receptors, namely NOD1 and NOD2, capable of sensing *C. trachomatis* and eliciting a subsequent activation of NF-kB pathway independent of other PRRs or MHC II-mediated stimulations (Mascellino, 2001; Derbigny *et al.,* 2005; Opitz *et al.,* 2005; Welter-Stahl *et al.,* 2006).

1.5 Immunogenetics

The course of infection with *C. trachomatis* is characterised by tremendous variation and heterogeneity between individuals (Land *et al.,* 2009; Morré *et al.,* 2009). The observed differences could partially be explained by bacterial virulence factors, as well as the environmental factors and patient's risk behaviour, however these only provide a limited account for the differences in infection rates, symptoms, clearace or severity (Land *et al.,* 2009) (Figure 2). A plethora of studies on other pathogens implicates the role of host immunogenetic factors in affecting the disparities observed (Martin & Carrington, 2005; Li *et al.,* 2005; Singh & Spector, 2009). These associations have been found by researching *C. trachomatis* as well. A study by Bailey and colleagues estimated that heritability contributes to differences in lymphoproliferative responses to ocular *C. trachomatis* infection with roughly 40% (Bailey *et al.,* 2009). What remains to be further elucidated is

which genes are responsible for these responses and how their different variants play a role. Nevertheless, the contribution of host's genomic factors to the clinical course of *C. trachomatis* infection has begun to be explained in the last decade (den Hartog *et al.,* 2006; Karimi *et al.,* 2009; Morré *et al.,* 2009; Ohman *et al.,* 2012).

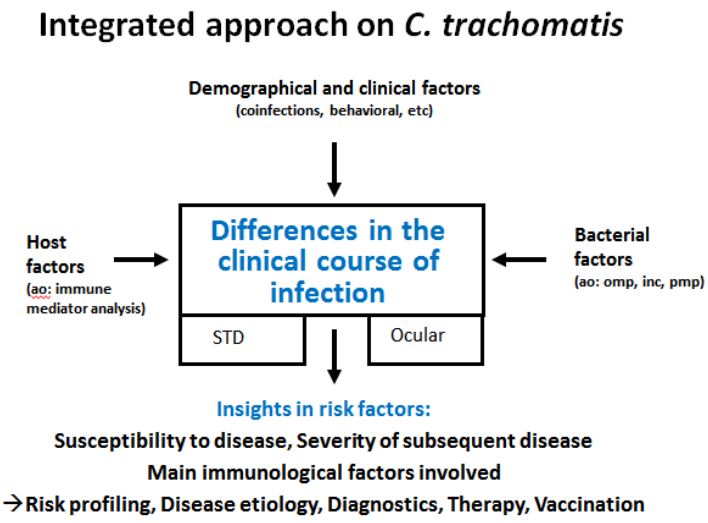

Figure 2. *The role of different factors associated with the risk and the clinical course of Chlamydia trachomatis infection*

A study by den Hartog and colleagues has shown that SNPs in PRRs TLR4, TLR9 and NOD2 individually increase the risk to develop tubal pathology after *C. trachomatis* infection (see Figure 3) (den Hartog *et al.,* 2006).

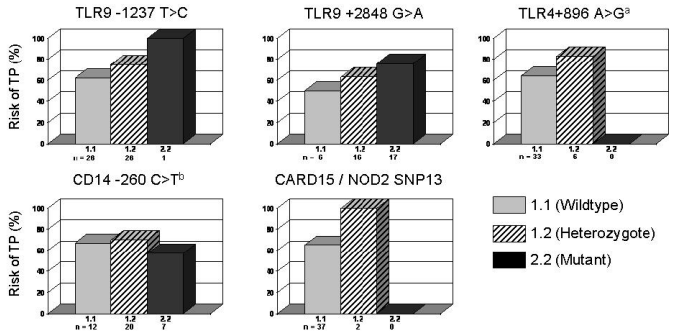

Figure 3. *The risk of tubal pathology (TP) in C. trachomatis IgG-positive subfertile women based on the genotype of different pathogen recognition receptor genes.* [a]*From Morré et al., 2003.* [b]*From Ouburg et al., 2005.*

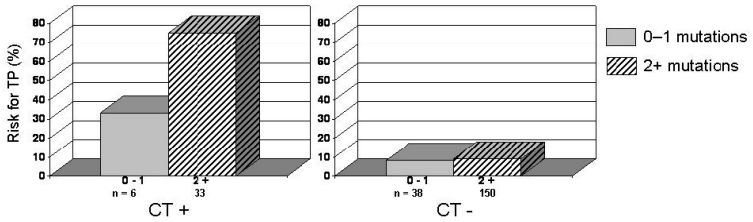

Figure 4. *The risk of tubal pathology (TP) in C. trachomatis IgG-positive (CT+) and IgG-negative (CT-) subfertile women increases with the number of SNPs in their PRR genes (adapted from den Hartog et al., 2006).*

Being a carrier of several rare alleles was more frequent (73% vs, 33%) in *C. trachomatis* positive women with tubal pathology compared to the *C. trachomatis* negative women. The same difference was not detected in *C. trachomatis* negative women with tubal pathology, indicating the importance of sensing of *C. trachomatis* in subsequent formation of an adequate immune response (den Hartog *et al.,* 2006) (Figure 4). This implies the major significance of initial recognition of *C. trachomatis* by receptors expressed in the genital tract, and is a crucial factor in protecting the host against tubal pathology.

Plausible hypothesis is that by elucidating the role of all the relevant host factors and combining them to other risk determinants (bacterial virulence and environment), better risk stratification could be made for the purpose of targeting women at highest risk of developing complications upon *C. trachomatis* infection. This would enable early prevention, reduction of unnecessary invasive diagnostic measures, avoiding the phychological burden and decreasing the costs. By further assessing the role of immunogenetic factors, their mutual associations, as well as interactions with other, non-

genetic factors, we would gain more insight into the disease mechanisms. It would enable us to more successfully conduct individual risk profiling and would therefore improve the efforts within health care in tackling *Chlamydia* infection and its consequences.

2. HPV

2.1 Epidemiology

Human papillomaviruses (HPVs) are a group of double-stranded DNA viruses which infect the cells of the basal layer of the epithelium (Villa *et al.,* 2006). Global HPV prevalence among women was estimated at 11,7%, in a large meta-analysis spanning five continents (Bruni *et al.,* 2010), attesting to the virus' status as the most ubiquitous sexually transmitted agent in the world. There is, however, an academic dispute on whether it is salient to refer to HPV as an STI, given the fact that successful transmission does not necessitate sexual contact. Furthermore, not all of the types have been associated with the infection of the genital regions. More than 150 HPV types have been identified by now, with new types being continuously characterised. Approximately 40 out of those have been found to infect the genital tract area (Stanley *et al.,* 2007; Chan *et al.,* 2012). These types can show preference for either skin or mucosal epithelia. Moreover, aside from differing in tropism, high-risk and low-risk subgroups can be distinguished based on each type's carcinogenic potential (Hazard *et al.,* 2006; Halec *et al.,* 2013), with several high-risk types showing preference for cervical compared to vaginal epithelium (D'Souza *et al.,* 2012) (Table 1).

Genus	*Tissue tropism*	*HPV types*		*Diseases*
Alpha	Mucosal and cutaneous	High oncogenic risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82	Intraepithelial neoplasia/ cervical, other anogenital, head and neck cancer
		Low oncogenic risk	6, 11, 42, 44, 51, 53, 83	Condylomata acuminata/ intraepithelial neoplasia/ musical lesions
			32, 42	Benign lesions
			3, 10, 28, 29, 78	More cutaneous, less musical lesions
			61, 62, 72, 81, 83, 84, 86, 87, 89	Benign mucosal lesions
			2, 27, 57	Common skin warts
			13, 26, 30, 34, 53, 54, 71, 74, 67, 69, 70, 85, 90	Musocal lesion
			7, 40, 43, 91	Mucosal and cutaneous lesions
Beta	Cutaneous	5, 8, 9, 12, 14, 15, 17, 19-23, 25, 36-38, 47, 80, 49, 75-76		Associated with epidermoplasia verruciformis
Gamma	Cutaneous	4, 48, 50, 60, 65		Cutaneous lesion
Mu	Cutaneous	1		Plantar wart
Nu	Cutaneous	63		Plantar wart

Table 1. *The overview of identified HPV types (adapted from Trottier et al., 2009).*

The relationship between high-risk types and different cancers has been well established over the past several decades. High-risk HPV types are routinely detected in 99,7% of all cervical cancers and are a confirmed etiological factor in the development of precancerous stages (cervical intraepithelial neoplasia, or CIN) as well as cervical cancer itself (zur Hausen 1996; Waalboomers et al 1999). Thirteen high-risk types have been identified to date, with 70% of cervical carcinoma cases being attributed to infections with types 16 and 18 (Petry *et al.,* 2013). These two types are also the most prevalent HPV types in women globally, with 3,2% and 1,4% prevalence for types 16 and 18, respectively (Forman *et al.,* 2012). The virus is necessary - but not sufficient - for the progression to an irreversible cancerous stage (Halec *et al.,* 2013). Vulvar, anal, penile and different cancers of head and neck also have HPV as a major factor in their etiology (Halec *et al.,* 2013; Forman *et al.,* 2012).

2.2 Treatment and prevention

The infection itself is typically asymptomatic. The progression of lesions may lead to an invasive cancer, which can be treated by radiotherapy and surgery. In the domain of prevention, on the other hand, considerable progress has been made in the past decade. In 2007, two vaccines – Gardasil and Cervarix – which reportedly immunise recipients against the two most oncogenic and most prevalent high-risk types, 16 and 18 (in case of Gardasil also against two low-risk, condyloma-inducing types, 6 and 11) have been approved for use (Casper *et al.,* 2008). The vaccines need to be administered prior to the first age of sexual contact, meaning before the first potential exposure to HPV, in order to reach its optimal protective effect. The implementation of the HPV vaccine into many countries' national vaccination programmes has been a milestone in the attempt to prevent cervical cancer, by targeting the two most carcinogenic HPV types. However, the true impact of this prevention intervention on HPV-caused cancers cannot be observed for a number of decades (Hull & Caplan, 2008; Carpenter & Casper, 2009). In Australia, however, significant declines in numbers of young women suffering from genital warts who were vaccinated in 2011 or earlier suggests that the human papillomavirus vaccine has a high efficacy (Ali *et al.,* 2013).

2.3 Pathogenesis and immune response

Carcinogenesis in HPV infection is an intricate process, which necessitates previous occurence of several genetic and epigenetic modifications (Hebner *et al.,* 2006; Lehoux *et al.,* 2009; Litjens *et al.,* 2013; Steenbergen *et al.,* 2014). The viral proteins are capable of inducing the changes leading up to host cell's neoplastic transformation. The viral genome

is composed of eight open reading frames encoded in the early region (E1, E2, E4, E5, E6 and E7) and in the late region (L1 and L2) (Burk *et al.,* 2009) (Figure 5).

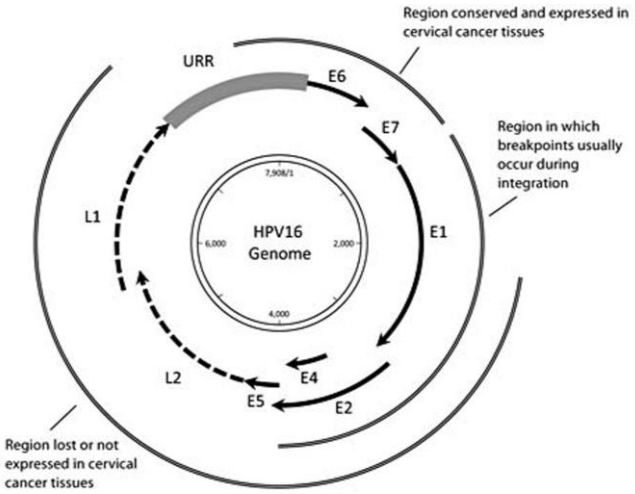

Figure 5. *Genome of the HPV virus (adapted from Burk et al., 2009).*

HPV genome also contains a third region, referred to as the long control region. The region is non-coding, but critical for transcription of the viral genes, the initiation of viral DNA replication, and the segregation of the viral genome in mitosis (Hebner & Laimins, 2006). In order to replicate its genome, HPV modulates the cell cycle, while deploying mechanisms to escape the host immune response, cellular senescence and apoptosis. As such, HPV infection leads directly and indirectly to genomic instability, further favouring transforming genetic events and progression to malignancy. Loss of control over viral oncogene expression in the basal cell layer, resulting in an increased production of E6 and E7, is considered as the critical event in the development of dysplastic lesions (Litjens *et al.,* 2013). The combined action of these two proteins results in uncontrollable proliferation and immortalisation and reduced apoptosis, which inevitably leads into chromosomal instability (Burk *et al.,* 2009). The viral genome frequently breaks at its fragile site within the E2 reading frame, resulting in a) higher likelihood of viral integration into the host genome, and b) loss of the suppressive function of E2 protein over the expression of E6 and E7 (Choo *et al.,* 2000).

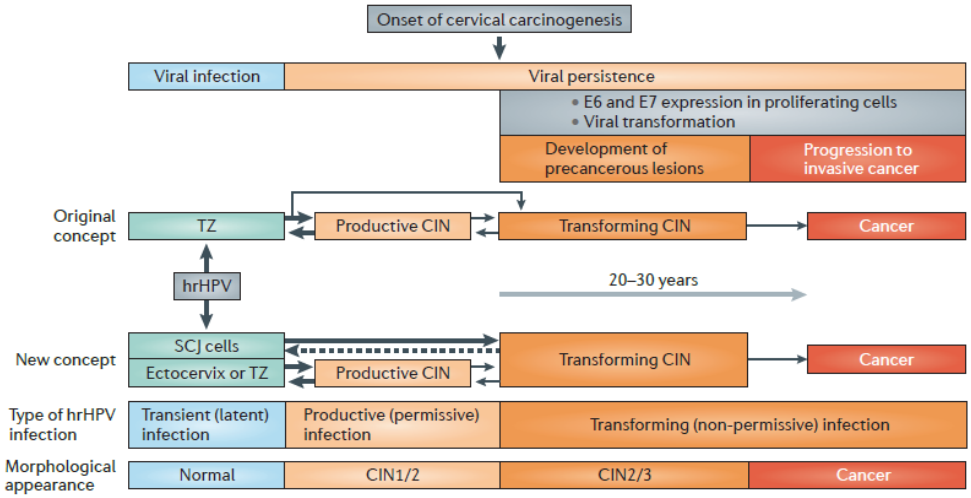

Figure 6. *HPV-mediated carcinogenesis – outcomes of exposure (taken from Steenbergen et al., 2014)*

Both viral and host factors may explain one's inability to clear the infection. One means by which HPV escapes immune detection is built into its natural life cycle (Lehoux *et al.,* 2009). Primary infection occurs in the basal cells of the stratified epithelium where viral genomes are maintained only at very low levels. Viral proteins are also very weakly expressed and confined mainly to the nucleus of basal cells. Increased protein expression only occurs as keratinocytes migrate through the upper layers of the epithelium where the adaptive immune system has limited access. Finally, newly assembled viral particles are released by natural shedding, a process that does not involve cell lysis, thereby preventing dendritic cell activation, pro-inflammatory cytokine liberation, and antigen presentation by Langerhans cells in the proximal layers of the epithelium (Kupper *et al.,* 2004; Tindle *et al.,* 2002). Interferon immunoregulatory effects were shown to be directly inhibited by HPV, both by reducing interferon expression and by interfering with its signalling pathways (Woodworth, 2002). Different viral types have acquired various, more specific ways of countering the immune surveillance (Lehoux *et al.,* 2009).

2.4 Immunogenetics and epigenomics

Understanding the interplay of immunogenetic factors is important for fully elucidating the role of immune response and inflammation in the aetiology of cervical cancer and other HPV-induced cancers. The pathobiology of these cancers is multifaceted and complex. Besides those factors that can block initial infections or affect the clearance of HPV infection, there are factors involved in promotion and progression of existing lesions (Burk *et al.,* 2009). Several PRRs (TLR9 for instance) appear to be directly involved in recognition of HPV, via its unmethylated CpG sites, and therefore the polymorphisms in these genes affect host's success in defending itself by eliciting an adequate immune response (Hasan *et al.,* 2007). So far, many studies contributed to our understanding of the role of host genomic factors in all of the above mentioned processes (Ivansson *et al.,* 2010; Rader, 2010; Stern & Einstein, 2010). **Chapter 3** of this thesis provides a comprehensive overview of empirical studies on immunogenetic polymorphisms associated with HPV and cervical carcinoma.

Epigenomics is a rapidly advancing field that contributes greatly to our understanding of diseases. Epigenomic alterations are essential for HPV-driven progression of neoplastic changes in cervical epithelial cells (Steenbergen *et al.,* 2014). Another promising aspect of epigenomics for future clinical management of infectious diseases is the (theoretical) ability to modify the biochemical changes occurring, thereby restoring gene expression. From the perspective of Public Health Genomics of infectious diseases, taking into account epigenomic information ought to enhance the disease model and advance successful translation into prevention, diagnostics and therapy.

3. Public Health Genomics and translational research

It was as early as 1963 when it was stated in a report by the World Health Organization's expert committee that taking genetics into account can add a "new dimension to public health work", which is to be of concern "not only for the health and well-being of persons now living", but also the future generations (http://www.who.int/genomics/history/en/). Back in 2005, an expert group defined Public Health Genomics in their Bellagio Report as "a multidisciplinary field concerned with the responsible and effective translation of genome-based knowledge and technologies for the benefit of population health" (Brand & in den Bäumen, 2007; Knoppers & Brand, 2008). The goal was to facilitate and optimise the uptake of the knowledge obtained from the Human Genome Project and subsequent endeavors and secure their implementation into healthcare (Khoury *et al.,* 2008; Burke *et al.,* 2010).

The term "bench to bedside" is used to reference the concept of 'translational medical research'. Many in the biomedical community see translational research as a way of thinking about and conducting life sciences research to accelerate healthcare outcomes. Basic scientists provide clinicians with new tools for use in patients and for assessment of their impact, and clinical researchers make novel observations about the nature and progression of disease that often stimulate basic investigations. Defined, translational research is "the process by which basic scientific discoveries are transformed through clinical application into new medical treatments and products to enhance the diagnosis, treatments, and prevention of diseases". Translational research is also concerned with how the process can be more efficient and accelerate the research process so discoveries can make more of an impact in the lives of patients (Khoury *et al.,* 2009).

An important reason why genome-based discoveries have not yet realised their clinical and public health potential is that attention and funding have been largely directed at the initial stage of scientific discovery, rather than extending it to the application, implementation, and evaluation stages (Burke *et al.,* 2010). This thesis attempts to make a contribution to the advancement of Public Health Genomics of STIs, namely *C. trachomatis* and HPV, by identifying genomic factors pertinent to infection with and progression of these diseases, and aid the efforts in successfully translating basic research findings into the clinical setting.

4. Socio-environmental factors

In this thesis, some of the socio-environmental components in relation to *C. trachomatis* and HPV infections have been explored in more depth. Research demonstrates that infectious diseases and STIs in particular have both a host genomic component to their susceptibility and severity (den Hartog *et al.,* 2006, Lal *et al.,* 2013), but that there is also a marked contribution by the environment (such as nutrition) and socio-cultural elements (for instance gender).

4.1 Gender

Research on gender disparities in health outcomes has begun to gain significant momentum only in recent years (Holdcroft, 2007; Klinge, 2007). The gradual abolishment of the traditional prototypical study subject – Caucasian male – has led to profound new insights into gender-specific risk exposures and subsequent differences in health (Epstein, 2004). Sexually transmitted infections were also found to have a strong gender component, in terms of gender both leading to different predispositions between groups and also STIs causing further disparities between groups, resulting in even more negative health consequences (Varga, 2003; Bermudez *et al.,* 2010; Valente & Auerswald, 2013).
The traditional focus on males in research has been absent for certain diseases. This has been observed by several authors and contradicts the prevailing notion that only women's health remains underexplored in research (Higgins *et al.,* 2010; Striegel *et al.,* 2012). The artefacts of the "white male effect" being increasingly recognised in recent years might be the underlying cause of this misperception (Finucane *et al.,* 2000; Epstein, 2004). HPV-caused male diseases for instance lack the attention in basic sciences, as does the research on prospective benefits of broadening the national HPV vaccination programmes to boys, given that current programmes in most countries target young girls only. There is a higher observance of several types of HPV-induced cancers in high-risk groups such as MSM (Daling *et al.,* 2004). Nevertheless, clinical studies still frequently tend to omit gender differences from their research focus and study designs, which also leads to women being at a disposition, to the potential detriment to their health (Marry Horrigan Connors Center report, 2014; http://www.wisdombog.com/pdf/11200415250.pdf). Gender can therefore play a role in one's access to healthcare, to the disadvantage of certain risk groups.
Men and women differ in susceptibilities to sexually transmitted diseases both due to biological (sex) and socio-cultural (gender) factors and their interactions. Genome-based knowledge is rapidly increasing, but sex and gender issues are often not explored (Addis, 2008; Kotz, 2014). Public Health Genomics would benefit from incorporating sex and gender analysis as its standard approach. It would help explain the manner in which gender and other socio-cultural factors modify genetic predispositions (Verdonk & Klinge,

2012).

5. Aims and scope of the thesis

The main aim of the thesis is to investigate the role of polymorphisms in genes involved in molecular recognition of STIs and subsequent immune and inflammatory responses, with a particular focus on *C. trachomatis* and HPV. Furthermore, the aim is to investigate the role and contribution of these polymorphisms in altering the risk of tubal damage and subfertility (in the case of *C. trachomatis*) and CIN and cervical cancer (as a consequence of a hrHPV infection). The thesis also covers the role of biobanking in genomic research of infectious diseases, as biobanks represent the cornerstones for researchers to have access to and utilise necessary samples. In order for the successful translation of genomic findings to take place, the process needs to start with basic scientists having access to adequate samples, which must be encompassed with clear regulations and governance of biobanks. The impact of gender as a confirmed, but insufficiently examined socio-economic factor contributing to the risk of HPV infection, HPV-related sequelae and prevention by vaccination has been addressed. Finally, the thesis summarises existing empirical knowledge of immunogenetic factors involved in *C. trachomatis* and HPV infections and their sequelae. Public Health Genomics is a field of research that comprises responsible and effective translation of genomic research results into health care applications. The field is redefining health paradigms by recognising the necessity of taking into consideration all individual characteristics and their interactions between genomic, biological and social determinants in order to fully comprehend disease aetiology and provide successful prevention, screening and treatment. The prerequisite for the advancement of personalised medicine approaches in infectious diseases is ensuring continuous research of host's and pathogen's factors, providing more robust efforts for translation of obtained knowledge and incorporating the neglected factors (such as gender and health literacy) more decisively into Public Health Genomics models.

Part I. Host genomic factors in infections with *Chlamydia trachomatis* and human papillomavirus

There is a considerable variation in women's individual responses to a *C. trachomatis* infection. In certain individuals, effective immune response eliminates the infection adequately without leaving tissue damage; however others can experience a persistent infection that may ascend upwards through the reproductive tract, heightening the risk of tubal damage and subfertility. The susceptibility, course and outcome of *C. trachomatis* infections depend on the interplay of socio-environmental factors, pathogen's virulence factors and host (immunogenetic) factors. Part II of this thesis features research that

corroborates earlier findings on the function of different immunogenetic polymorphisms on *C. trachomatis* infection susceptibility and severity, but also seeks to identify novel immunogenetic factors associated with *C. trachomatis* infection in women. The goal is to advance host genomic field and provide the impetus for effective translation into health care.

In **Chapter 1**, we aim to elucidate the precise involvement of two distinct functional polymorphisms in two different NOD-Like Proteins (NOD1 and NOD2) in susceptibility to and severity of *C. trachomatis*. NOD receptors are already known to be involved in recognition of intracellular pathogens, including *Chlamydiae*. This study attempts to expand on the already known roles of these polymorphisms for the first time in *C. trachomatis* infection.

Chapter 2 aims to assess the role of several polymorphisms in genes within the vitamin D metabolic pathway in susceptibility to and severity of *C. trachomatis* infections. There are a number of studies that already indicate these polymorphisms in immune responses to other infections. We aim to add to this body of knowledge in order to try to confirm these effects on *C. trachomatis* infection.

In **Chapter 3**, our aim is to provide a comprehensive summary of the identified host immunogenetic factors of HPV infection and cervical cancer etiology. By organising the genes into specific categories based on the function of their products, we aim to systematise the empirical knowledge published to date.

Part II. Translation of host genomics from bench to bedside – a two-way street

Scientists are mindful that the bench-to-bedside approach to translational research is a two-way street. An effective translation of relevant basic research results must be preceded by access to biological samples, which ought to be easily obtained from biobanks. Hence, bodies governing biobanks need to ensure clear and simplistic regulatory procedure regarding the manner in which researchers can get a hold of required samples. Ultimately, the process should result in successful clinical applications that will enable better risk group stratification, ensuring a more effective patient management.

In **Chapter 4**, the aim is to provide insights into the relevance of biobanking in infectious disease research. The role of biobanking in infectious disease genomics is discussed, together with examples of infectious disease biobanks with transparent procedures and high publication visibility. The potential main stumbling stones in current usage and access to infectious disease biobanks are given.

Chapter 5 aims to provide an overview of known immunogenetic factors of HIV, *C. trachomatis* and HPV infection, and describes their translational potential into clinics. An

insight into a prospective advancement in tubal pathology and subfertility diagnostics has been outlined in this chapter as well.

Part III. Socio-cultural factors and health literacy – neglected areas in host-pathogen research, diagnostics and translation

The outcome of a hrHPV infection may greatly differ between individuals, due to the way in which various factors combine and interact in each individual. As is the case with *C. trachomatis*, immunogenetic aspects of HPV infection are bolstered by empirical studies and are in fact characterised by a extensive body of research. The contribution of environment, for instance socio-cultural factors, such as gender in HPV infection as well as its related diseases has been established, however the exact involvement of these elements requires further elucidation. In recent years we have witnessed the advent of a novel means of prevention in the form of bivalent and tetravalent vaccines. Early studies attest to the effectiveness of these vaccines in precluding the infection with designated HPV types (as well as a likely partial protection against other types of HPV viruses).

However, the uptake and acceptance of the vaccines also appear to be contingent upon socio-cultural factors, which are addressed in **Chapter 6**. In this chapter we aim to categorise the published state-of-the-art knowledge on the role of gender as a socio-cultural determinant in explaining the risk of contracting HPV.
Chapter 7 aims to provide insight into relevance of genome-based health literacy. Additionally, we will describe the issue of the input of genome-based knowledge into clinics from the perspective of host genomics and its applications into that setting. More specifically, it is important to investigate what levels of knowledge and attitutes exist in respect to the implementations of this knowledge into health care, from the perspective of both health specialists (*e.g.* gynaecologists) and the patients/users.

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PART I

HOST GENOMIC FACTORS IN INFECTIONS WITH *CHLAMYDIA TRACHOMATIS* AND HUMAN PAPILLOMAVIRUS

CHAPTER 1

NOD1 in contrast to NOD2 functional polymorphism influences *Chlamydia trachomatis* infection and the risk of tubal factor infertility

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Submitted.

Abstract

Intracellular pattern-recognition receptors NOD1 and NOD2 are capable of sensing common structural units of bacterial walls. Recognition triggers specific immune signaling pathways and leads to pro-inflammatory cytokine upregulation and adequate immune response. We investigated whether two functional polymorphisms in *NOD1* and *NOD2* exert an effect on susceptibility to (STD patients) and severity of (female patients visiting the Fertility clinic) *Chlamydia trachomatis* infection in 807 Dutch Caucasian women. A significant association of the *NOD1* +32656 GG insertion variant with protection against infection with *C. trachomatis* has been detected [p: 0.0057; OR: 0.52]. Carriers of this variant were, however, at a heightened risk of tubal factor infertility (TFI) [p: 0.038; OR: 2.25]. When comparing *C. trachomatis* positive women without symptoms, to *C. trachomatis* positive women with symptoms, to *C. trachomatis* positive women with TFI, we observed an increasing trend in carriage of the GG allele [P_{trend}: 0.0003]. *NOD2* 1007fs failed to reveal an association. We hypothesise that the underlying mechanism might be a functional effect of the GG insertion on IFN-beta-dependent regulation of immune response in the genital tract. The research is part of an ongoing effort of identifying key polymorphisms that determine the risk of TFI and effectively translating them into the clinical setting for the purpose of optimising diagnostic management of women at risk for developing TFI.

Introduction

Chlamydia trachomatis is the most common sexually transmitted bacterial infection, and it is strongly associated with tubal infertility (Brunham *et al.,* 1985; Sellors *et al.,* 1988; Mabey, 2014). The course of *C. trachomatis* infection varies between individuals - only certain people are successfully infected and only part of those infected develops more severe disease as a result of an uncleared infection and prolonged accompanying inflammation (Kinnunen *et al.,* 2002; Darville & Hiltke, 2010). Substantial evidence for the contribution of variation of host immunogenetic factors in the clinical course of infection with this pathogenic bacterium has been accumulated, mainly in the last decade (Wang *et al.,* 2005; den Hartog *et al.,* 2006; Bailey *et al.,* 2009; Morré *et al.,* 2009; Jiang *et al.,* 2012; Al-Kuhlani *et al.,* 2014). These factors appear at this point to be the most promising biological indicators of complicated infection with *Chlamydiae* (Ouburg *et al.,* 2009; Lal *et al.,* 2013; Malogajski *et al.,* 2013; Branković *et al.,* 2014).

Nucleotide-binding oligomerisation domain (NOD) receptors are intracellular proteins that participate in diverse innate immune processes (Proell *et al.,* 2008). Receptors NOD1 and NOD2 were found to play a crucial role in priming immune responses to intracellular pathogens (Inohara *et al.,* 2001; Chamaillard *et al.,* 2003; Inohara & Nunez, 2003), but also in initiating autophagy of infected cells, thereby demonstrating their multifaceted role in host defence (Cooney *et al.,* 2010; Travassos *et al.,* 2010; Homer *et al.,* 2012). Research consistently shows evidence of the involvement of NOD receptors and their polymorphisms in a multitude of diseases - various infections, inflammatory bowel disease (IBD), asthma, as well as cancers (Lu *et al.,* 2010; McGovern *et al.,* 2005; Oosting *et al.,* 2010; Wang *et al.,* 2012). NOD1 (also known as CARD4) and NOD2 (CARD15) are the most extensively researched members of the NLR family (Correa *et al.,* 2012). NOD1 recognises gamma-d-glutamyl-*meso*-diaminopimelic acid, specific for peptidoglycans found predominantly in walls of Gram negative bacteria (Chamaillard *et al.,* 2003; Uehara *et al.,* 2006). NOD2 is capable of sensing muramyl dipeptide, a minimal peptidoglycan motif ubiquitous in walls of both Gram negative and Gram positive bacteria, making it a more general pathogen detector (Chamaillard *et al.,* 2003; Girardin *et al.,* 2003). Both receptors signal the presence of lipopolysaccharides (LPS), found in outer membranes of Gram negative bacteria (Inohara *et al.,* 2001; Marriott *et al.,* 2005). Expression of NOD2 is mainly observed in monocytes (Bonen & Cho, 2003), while NOD1 appears to be active in a wider range of cell types that represent potential points of first entry for pathogens (Park *et al.,* 2007; Kufer *et al.,* 2008).

Activation of the Pathogen Recognition Receptor (PRR) signalling pathways results in NF-κB activation and subsequent immune response (Takeuchi & Akira, 2010). A number of studies have implicated the involvement of NOD1- and NOD2-induced immune responses in genital tract infection with *C. trachomatis* (Derbigny *et al.,* 2005; Opitz *et al.,* 2005;

Welter-Stahl *et al.,* 2006). However, whether functional polymorphisms of NOD1 and NOD2 impact *C. trachomatis* infection and clinical course has hardly been explored. One study investigated the role of *NOD2* 1007fs polymorphism in tubal pathology upon *C. trachomatis* infection and reported a tendency for the heterozygotes, however the numbers were too small and mutant homozygotes were not present among samples (den Hartog *et al.,* 2006). *NOD1* +32656 T>GG deletion-insertion polymorphism affects the receptor's recognition of bacterial motifs. Different studies associated each of its alleles with a diverse set of diseases. GG insertion variant appeared to confer higher susceptibility to erosive oesophagitis in *Helicobacter pylori*-infected individuals (Oikawa *et al.,* 2012) and was associated with asthma in Australian individuals (Hysi *et al.,* 2005). Deletion allele (T) was found to heighten the risk of IBD's early onset in British Caucasian population (McGovern *et al.,* 2005).

To our knowledge, the role of the *NOD1* +32656 T>GG and *NOD2* 1007fs polymorphisms in susceptibility to *C. trachomatis* infection and the severity, i.e. the risk of developing tubal factor infertility (TFI) has not been studied before. We hypothesise that NOD1 and NOD2 proteins might play a role in the course of *C. trachomatis* infections. To analyse this we used two clinically well-defined Dutch Caucasian cohorts.

Methods

Study population

Susceptibility cohort. Out of 1150 female patients visiting the STD outpatient clinic in Amsterdam, The Netherlands (between 2000-2002), we selected all Dutch Caucasian women (n=737, age 18-30). Questionnaires were collected in regards to urogenital complaints, varying from increased discharge, having bloody discharge during and/or after coitus, recent lower abdominal pain (not gastrointestinal or menstruation-related) and/or dysuria. A flow diagram of the cohort used for the current study is presented in panel A of figure 1. All participants signed informed consent forms. The Medical Research Involving Human Subjects Act (WMO, Dutch Law) stated that official approval of the study by the Medical Ethical Committee does not apply to our anonymous human material collected (MEC Letter reference: # 10.17.0046). The local medical ethical committee also approved this study, based on the fact that in the Netherlands ethical approval is not required for a retrospective use of de-identified clinical samples. Nevertheless, since we performed host genetic marker studies in relation to Chlamydial infection, we made sure all participants signed informed consent forms.

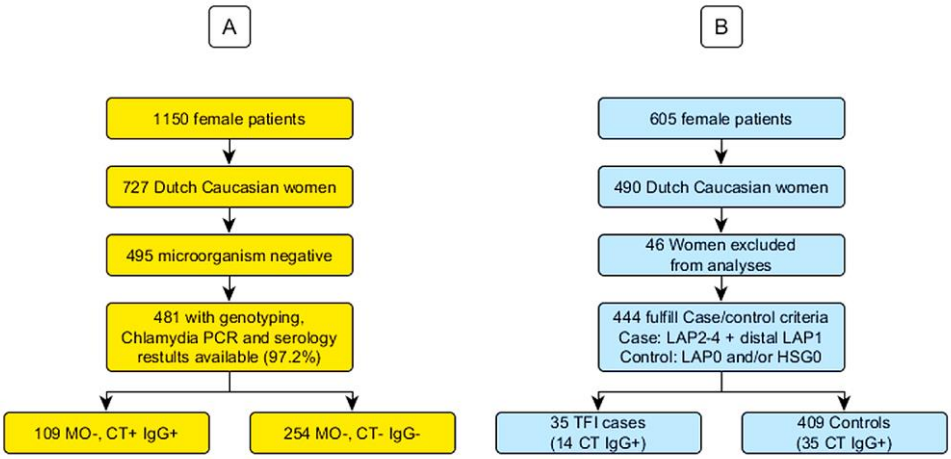

Figure 1. *Flow-chart overview of the cohort selection, sample numbers and microbiology, C. trachomatis DNA analysis and IgG serology analysis. A. susceptibility cohort; B. severity cohort. (CT+ = positive for C. trachomatis DNA; CT- = negative for C. trachomatis DNA; IgG+ = positive for C. trachomatis-specific IgG antibodies; IgG- = negative for C. trachomatis-specific IgG antibodies; MO- = microorganism (N. gonorrhoeae, T. vaginalis, HSV1/2, C. albicans) negative; TFI+ = positive for TFI; TFI- = negative for TFI).*

Severity cohort. Our severity group consisted of 605 female patients visiting the Fertility clinic in the University Medical Center Groningen (UMCG) between 2007-2012. In all women Chlamydia IgG antibody testing (CAT) and hysterosalpingography and/or laparoscopy had been part of the fertility work-up. Data were retrospectively collected by chart review and anonymised for the researchers. Among the 605 subfertile patients (age 20-41), 490 were Dutch Caucasian. Two independent investigators, who were unfamiliar with the CAT results, scored the laparoscopy reports to assess the grade of tubal pathology (0 = no abnormalities; 1 = any peritubal and/or periovarian adhesions and/or proximal or distal occlusion of at least one tube; 2 = extensive periadnexal adhesions and/or proximal or distal occlusion of at least one tube; 3 = extensive periadnexal adhesions and/or distal occlusion of at least one tube; 4 = extensive periadnexal adhesions and/or distal occlusion of both tubae).

The hysterosalgography results were scored as 0 = normal; 1 = unilateral occlusion proximal; 2 = unilateral occlusion distal; 3 = bilateral occlusion proximal; and 4 = bilateral occlusion distal. For the present study TFI was defined as extensive peri-adnexal adhesions and/or distal occlusion of at least one tube at laparoscopy (Laparoscopy Grades 1 Distal, 2, 3 and 4). By applying this definition, 35 cases with TFI were identified. Controls were defined as women negative for TFI (as determined by laparoscopy (grade 0 and/or

hysterosalpingography grade 0: n=444). Women who did not fulfill the criteria or had missing data for cases and controls were excluded from the study (n=46). A flow diagram of the cohort used for the current study is presented in panel B of figure 1.
In the Netherlands, no ethical board approval is required for retrospective chart review and collection of anonymised data. Couples attending the Fertility clinic in the UMCG are informed about possible use of their anonymised data for research purposes, and a "no objection procedure" is followed. Only patients who had not objected were included in the present study. From those patients available clinical material (sera) was used. No additional or new clinical material was collected for the purpose of this study.

Laboratory analyses

Susceptibility cohort

A cervical swab was taken for the detection of *C. trachomatis* DNA using PCR. Peripheral venous blood was collected for the analysis of IgG antibodies against *C. trachomatis*. Infections with the microorganisms *Candida albicans, Neisseria gonorrhoeae, Trichomonas vaginalis,* or herpes simplex virus (HSV) 1 or 2 may result in symptoms similar to *C. trachomatis* infection, therefore the patients' infection status for these microorganisms has been determined (van Doornum *et al.,* 2001). Only the patient samples negative for all of these microorganisms were used in our analyses. In addition, *C. trachomatis* serovars were assessed in all *C. trachomatis* positive samples, as described previously, to confirm true *C. trachomatis* positivity (Morré *et al.,* 1998).

Severity cohort

Blood had been drawn from all patients for *Chlamydia* IgG antibody testing (CAT), *C. trachomatis* IgG MOMP serology was determined by Medac *C. trachomatis* IgG p Elisa (Medac, Germany).

SNP detection

Two polymorphisms genotyped in this study were *NOD1* T/GG (+32656 T>GG, partially identified as rs6958571) and *NOD2* 1007fs (rs2066847, SNP13, Leu1007fsinsC, 2936insC). We used an assay-by-design from Applied Biosystems to detect the *NOD1* +32656 T>GG polymorphism. Detection of the *NOD2* 1007fs mutation was performed using real-time PCR under standard conditions.

Statistical methods

We defined the following groups:

Susceptibility:
1) C*. trachomatis* positive: Women who are both *C. trachomatis* DNA and IgG positive; 2) *C. trachomatis* negative: Women who are both *C. trachomatis* DNA and IgG negative; 3) *C. trachomatis* positive with or without symptoms. In all groups women infected with other microorganisms were excluded from the analyses to generate clear groups for the association between symptoms or the lack of symptoms and the SNPs analysed.

Severity:
1) *C. trachomatis* positive and negative respectively: CT IgG serology positive or negative women respectively; 2) TFI positive and negative: Tubal factor infertility positive or negative women; 3) *C. trachomatis* positive with TFI: CT IgG serology positive women with tubal factor infertility; 4) *C. trachomatis* positive without TFI: CT IgG serology positive women without tubal factor infertility. Before conducting the severity analyses, the CT IgG positivity in cases and controls was assessed to show the expected relation between CT serology positivity and the severity of tubal pathology. Finally, trend analyses were performed to test the hypothesis the polymorphisms would show a trend when comparing CT+ women without symptoms to those with symptoms to those with TFI.

All groups were tested for Hardy-Weinberg equilibrium to check for Mendelian inheritance. Fisher's exact and $\chi 2$ tests were used where appropriate and p-values <0.05 were considered statistically significant.

Results

Genotype distributions were in Hardy Weinberg Equilibrium.
Full overview of genotype distributions for all the subgroups based on the *C. trachomatis* status and their presence or absence of symptoms are presented in Table 1.

	NOD1 +32656T/GG							*NOD2* 1007fs insC						
Group	n	T/T	%	T/G G	%	GG/ GG	%	n	-/-	%	-/C	%	C/C	%
CT+	109	73	67%	32	29%	4	4%	109	105	96%	4	4%	0	0%
CT+ S	50	27	54%	20	40%	3	6%	50	47	94%	3	6%	0	0%
CT+ AS	59	46	78%	12	20%	1	2%	59	58	98%	1	2%	0	0%
CT-	254	130	51%	115	45%	9	4%	254	245	96%	9	4%	0	0%
TFI+	32	13	41%	18	56%	1	3%	35	35	100%	0	0%	0	0%
TFI-	391	237	61%	134	34%	20	5%	405	393	97%	10	2%	2	0%
CT+ TFI+	13	6	46%	7	54%	0	0%	14	14	100%	0	0%	0	0%
CT+TFI-	33	19	58%	13	39%	1	3%	35	35	100%	0	0%	0	0%

Table 1. *Distribution of the NOD1 +32656 T>GG and NOD2 1007fs genotypes in Dutch Caucasian women (negative for tested non-C. trachomatis microogranisms) with or without clearly defined C. trachomatis infection (CT+ = positive for C. trachomatis DNA and C. trachomatis-specific antibodies; CT- = negative for C. trachomatis DNA and C. trachomatis-specific IgG antibodies; N = number of samples with both serological and genotyping results; S = symptomatic; AS = asymptomatic; TFI = TFI).*

Susceptibility

NOD1 +32656 T>GG

Carriage of the *NOD1*+32656 GG insertion was significantly lower in *C. trachomatis* positive women (33%) compared to *C. trachomatis* negative women (49%) [p: 0.0057, OR: 0.52, 95% CI: 0.32 – 0.83] (Table 1). *C. trachomatis*-positive women carrying the GG insertion were more likely to have symptoms [p: 0.013, OR: 3.01, 95% CI: 1.32-6.91]. Homozygous GG carriage increased risk of symptoms [OR: 3.70] in CT positive women, although this did not reach statistical significance.

NOD2 1007fs

Carriage of the *NOD2* 1007fs polymorphism did not show a significant association with susceptibility to *C. trachomatis* infections (Table 1). Carrying the C insertion allele and symptoms in *C. trachomatis* positive women showed a risk effect similar to that observed in *NOD1* *GG carriage [OR: 3.70], although this did not reach statistical significance.

Severity

Presence of *C. trachomatis* IgG antibodies significantly increased in more severe tubal factor infertility [Figure 2; P_{trend}: <0.0001], confirming the expected relation between past *C. trachomatis* infections and development of tubal pathology.

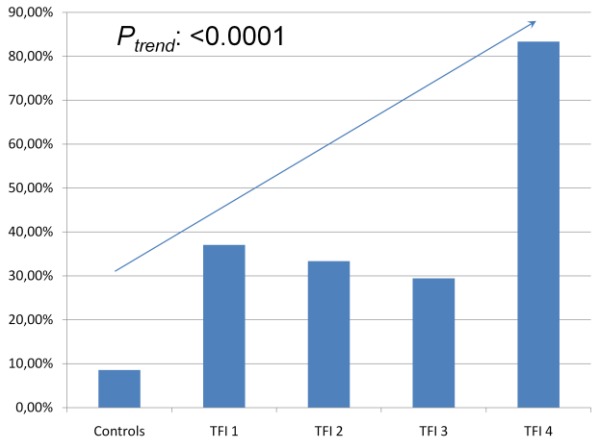

Figure 1. *Distribution of C. trachomatis positivity in controls and TFI 1-4 groups (with increasing severity of TFI). In all other analyses in this study TFI was defined as extensive peri-adnexal adhesions and/or distal occlusion of at least one tube at laparoscopy (Laparoscopy Grades 1 Distal, 2, 3 and 4).*

NOD1 +32656 T>GG

Carriage of the *NOD1*+32656 GG insertion was significantly more frequent in women diagnosed with TFI (59,4%) compared to women negative for TFI (39,4%) [p: 0.0383, OR: 2.25, CI: 1.08-4.69].
When comparing *C. trachomatis* positive women without symptoms, to *C. trachomatis* positive women with symptoms, to *C. trachomatis* positive women with TFI, we observed an increasing trend in carriage of the *NOD1* GG allele (Figure 3; P_{trend}: 0.0003).

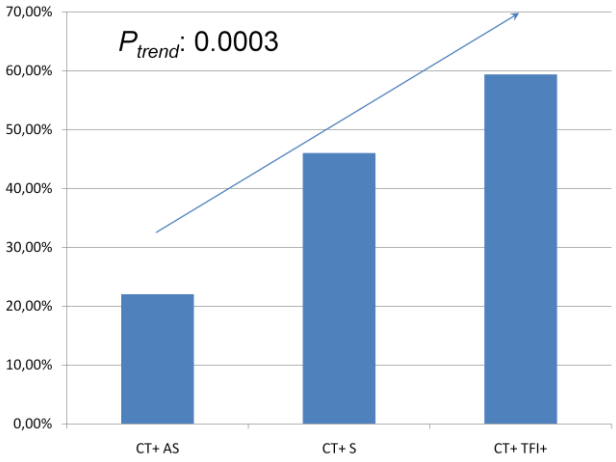

Figure 2. *Carriage of the NOD1 *GG allele in C. trachomatis positive women without symptoms (AS), with symptoms (S), and with tubal factor infertility (TFI).*

Discussion

Our results show a significantly reduced carriage of the *NOD1* +32656 GG insertion allele in *C. trachomatis*-positive women compared to the *C. trachomatis*-negative women, indicating a protective effect against *C. trachomatis* infections. There was a significant association of GG carriage with the occurrence of symptoms during an infection. Presence of symptoms was also significantly more frequently seen in *NOD1* GG homozygotes among *C. trachomatis* positive women. The *NOD2* C insertion allele and symptoms in *C. trachomatis* positive women showed the same direction of association.

Analysis of our severity cohort revealed, however, a deleterious effect of the GG insertion polymorphism in patients with TFI. Unlike in the susceptibility cohort, it was significantly more frequent in *C. trachomatis*-specific IgG-positive women diagnosed with TFI compared to women negative for *C. trachomatis*-specific IgG antibodies. *NOD2* 1007fs did not show associations with either susceptibility to *C. trachomatis* infection or the subsequent TFI in our study.

Our results contribute to the incomplete body of knowledge on the role of NOD1 receptor in infections with *C. trachomatis*. This is, to our knowledge, the first study of the effects of *NOD1* +32656 T>GG in *C. trachomatis*. Despite of the fact that several studies confirmed that NOD1 indeed senses and induces cellular changes in response to this pathogen, precise mechanisms and scope of its involvement in this particular infection are not fully

understood (Welter-Stahl *et al.,* 2006). It has been established that an upregulation of pro-inflammatory cytokine genes upon *Chlamydiae* infection occurs in mice primary fibroblasts, but not in those from NOD1-deficient mice. The study did not confirm that the presence of a functional NOD1 receptor also increased cytokine secretion in *C. trachomatis* infection (Welter-Stahl *et al.,* 2006). Opitz and colleagues demonstrated that *Chlamydia pneumoniae* induced a NOD1- (and NOD2-) mediated NF-κB activation in HEK293 cells. In endothelial cells, NOD1 played a dominant role in triggering a *C. pneumoniae*-mediated inflammatory process (Opitz *et al.,* 2005). Another study has shown that NOD1 and NOD2 are expressed in a cloned murine fallopian tube epithelial cell line (Derbigny *et al.,* 2005). What lacked so far are studies investigating the exact associations of different functional *NOD* polymorphisms in relation to infection with *Chlamydiae*.

In vitro studies of NOD1 recognition of pathogenic bacteria and subsequent cytokine induction point to its role in maintaining intestinal defence against invasive Gram negative species, but their precise role is not always as clear in *in vivo* models (Kim *et al.,* 2004; Travassos *et al.,* 2005).

In a study of immune responses to *Chlamydia muridarum* in cloned murine oviduct epithelial cell lines, the increase in acute phase cytokine secretion was driven mainly by TLR2 activation (Derbigny *et al.,* 2005). This might indicate a less crucial role of NOD proteins in tubal pathology than they appear to have in susceptibility to *Chlamydiae.* Nevertheless, the findings of our study might challenge that assumption.

NOD1 has been identified as the maximal inducer of the secretion of interferon-beta (IFN-beta), typical of murine genital *C. muridarum* infection (Prantner *et al.,* 2010). Research on the effect of IFN-beta shows its pleiotropic nature and variability across different bacterial infections and in different tissues. The effect of IFN-beta in *Chlamydia*-infected mice is, however, adverse – secretion of IFN-beta results in the inhibition of *Chlamydia*-specific CD4 T-cell responses and the delay of *C. muridarum* clearance (Nagarajan *et al.,* 2008). Type I interferons (IFN-alpha and IFN-beta) were also demonstrated to enhance susceptibility to *Listeria monocytogenes* (Auerbuch *et al.,* 2004; Carrero *et al.,* 2004; O'Connell *et al.,* 2004). However, in a study of a lung model infected with *C. pneumoniae*, IFN-beta appeared to be acting synergistically with IFN-gamma and helped resolve the infection (Rothfuchs *et al.,* 2006). Similar was found in *H. pylori* infection in a murine model, where IFN-beta enhanced Th1 differentiation as a result of NOD1 stimulation, which in turn led to a heightened IFN-gamma secretion (Watanabe *et al.,* 2010).

Hence, the protective effect of the *NOD1* +32656 GG insertion variant on susceptibility to *C. trachomatis* encountered in our study might be consistent with the findings of these studies. Insertion would then likely disrupt the native functionality of NOD1, leading to diminished IFN-beta secretion. This would prevent its inhibition of *Chlamydia*-specific T-cell responses, thereby resulting in an effective immune reaction to the infection and a

more rapid clearance. Specific IFN-gamma-producing T-cells can successfully clear *C. trachomatis* infection (Miyairi *et al.,* 2010), hence it is relevant how a NOD1-dependent immune response might influence this type of immune response. Conversely, in women with TFI, a full-fledged immune reaction in the upper reproductive tract may damage the tubae, therefore the ascended spreading of *C. trachomatis* in the reproductive tract would put the female GG allele carriers in a greater risk of TFI. This would explain how a same variant may lead to different consequences of the infection in lower and upper genital tract. Higher occurrence of symptoms in carriers of the GG insertion would also be consistent with this hypothesis – a more elaborate immune reaction to *C. trachomatis* would manifest in the form of more pronounced symptoms. Also, the fact that different CD4 T cell subsets take dominance in different parts of the genital tract in response to *C. trachomatis* infections may also be contributing to the diversity in outcomes of the infection (Marks *et al.,* 2010). A number of studies used to construct our hypothesis has however been done on murine models. Further research on the roles of the *NOD1* +32656 T>GG polymorphism, IFN-beta secretion, specific T-cell function in relation to human urogenital *C. trachomatis* information is therefore needed.

Moreover, *NOD1* +32656 GG insertion appears to not only alter the sensing capacity of NOD1. It auto-modulates the translation of the protein, with GG/GG carriers expressing much lower NOD1 levels compared to T/T carriers (Oikawa *et al.,* 2012). Considering the proposed effect of NOD1-induced IFN- beta secretion on *C. trachomatis* infection in urogenital tract, the insertion would therefore exert protection against the infection via two mechanisms. This warrants further studies on the NOD1-induced immunity in response to urogenital *C. trachomatis.*

The research on the role of NOD1 variants has been more extensive in cases of non-communicable diseases, and might be beneficial in further explaining our findings. Empirical evidence of the involvement of *NOD1* +32656 polymorphism in the etiology and pathophysiology of chronic inflammatory diseases is more abundant, however occasionally inconsistent and contradictory (van Limbergen *et al.,* 2006; meta-analysis by Lu *et al.,* 2010). A meta-study by Lu and colleagues (2010) of the effects of the mutant variant *NOD1* +32656 effect in IBD concluded that the GG insertion acts protectively in Caucasian populations, however only in the group with age of onset under 40. The polymorphism is in the non-coding region, near the beginning of intron IX, or precisely 34 bp from the intron IX– IX boundary. Exon IX encodes the LRR (leucine-rich repeat), a segment important for NOD1 function, therefore the LRR content of the splicing variant might be affected (Hysi *et al.,* 2005). The precise mechanisms by which the polymorphism affects different diseases have not yet been elucidated, but the assumption is that polymorphisms in this region result in changes in expression of naturally occurring NOD1 splice isoforms (Girardin *et al.,* 2005; Lu *et al.,* 2010; Corridoni *et al.,* 2014). Different diseases might associate with different isoforms resulting from differential splicing.

Alternatively, different polymorphism variants might also lead to altered binding of molecules interacting with NOD1 or resulting in an abnormal gene expression, which are all plausible even with non-coding polymorphisms such as +32656 T>GG (Lu *et al.,* 2010). A way to explain the role of NOD1 and its polymorphisms would be to further research on these different splicing forms and how they relate to alterations in sensing.

Analysis of *NOD2* 1007fs in susceptibility to and severity of *C. trachomatis* infection did not demonstrate an association in our study. Therefore, we did not manage to confirm the assumption that this functional polymorphism profoundly affects the *C. trachomatis* sensing capabilities. Den Hartog and colleagues observed a trend of *NOD2* 1007fs carriage in tubal pathology patients, but the sample numbers were too small for statistical associations (den Hartog *et al.,* 2006).

NOD2 mutations resulting in truncated LRR regions lead to Crohn's disease, arguably due to the impaired sensing of pathogens and disruption in the intestinal protection barrier, followed by invasion of the intestinal lining and prolonged inflammation, leading into IBDs (Li *et al.,* 2004; Vignal *et al.,* 2007). The inability of monocytes to register pathogens might ultimately result in an exaggerated adaptive response (Ogura *et al.,* 2001). However, how the mutations in the LRR-coding section affect their carriers in the presence of *C. trachomatis* infection has not been extensively studied to date.

It should be noted that the *NOD2* 1007fs mutant allele is rare in the general population. In our susceptibility cohort, there were no patients homozygous for this allele, and only 4% heterozygotes were observed. The severity cohort had only 0.49% mutant homozygotes. We did not observe any effects of mutation carriage when comparing *C. trachomatis* cases versus controls, nor in comparisons based on the presence or absence of symptoms within these two groups. Providing the *NOD2* 1007fs mutation was recessive in its nature, the absence of any observed effect in a *C. trachomatis* positive cohort that lacks the homozygotes would not be surprising, since it could be compensated in heterozygote's phenotype by the wildtype allele. However, several previous studies have found that the compound heterozygote carriage for this and one other polymorphism (SNP12) resulted in a heightened risk of developing Crohn's disease (Hugot *et al.,* 2001; Heresbach *et al.,* 2004). Even though we failed to confirm the association of *NOD2* 1007fs in our study, we encourage further research involving a larger study group, preferably with more other polymorphisms analysed simultaneously.

Given the partially converging roles of NOD1 and NOD2 in sensing certain pathogens, it can be useful to consider investigating whether combinations of their polymorphisms exhibit synergistic effects. Therefore a greater number of polymorphisms would need to be researched. There is a potential for implementing the knowledge on these factors and using them as genetic traits in order to improve individual risk predictions by existing clinical models, as part of the paradigm shift within health care towards ppersonalised medicine (Harvey *et al.,* 2012; Lal *et al.,* 2013; Malogajski *et al.,* 2013). More work in this

field, however, still has to be done. A study by Sanders and colleagues (2013) increased the predictive capacities of a post-meningitis hearing loss prediction model by adding a number of single nucleotide polymorphisms (SNPs) in different PRR genes (Sanders *et al.,* 2013). Women carrying two or more SNPs in PRR genes involved in recognising *C. trachomatis* show more than two-fold higher risk of developing tubal pathology after *C. trachomatis* infection compared to women with less than two PRR SNPs (den Hartog *et al.,* 2006). The studies show the potential of host genetic markers as indicators of risk of complication from *C. trachomatis* infection in women. Host genetic markers could be applied for improving clinical management of women at risk and making more salient decisions on which patients to refer to laparoscopies for diagnosing tubal pathology, which are invasive and costly diagnostic procedures.

In order to identify immunogenetic factors with strongest predictive value, next-generation sequencing and genome-wide association studies (GWAS) are a favourable alternative and can be used next to the candidate gene approach. At this year's Thirteenth International Symposium on Human Chlamydial Infections, two important preliminary studies in the field were presented. Roberts and colleagues (2014) are the first ones to use the GWAS approach to screen for pathway-wide genomic differences comparing cases of scarring trachoma with controls. They used Pathway of Distinction Analysis (PODA) to test for associations between groups of SNPs, in which they focused on identifying functional gene and gene-to-pathway associations. The ongoing study will be extended by validation in a second case-control cohort and by further in vitro analysis and system biology based analyses. The second study was presented by Su and colleagues (2014) who used an advanced recombinant inbred mouse strain set to identify sets of genes associated with disease severity phenotypes with special attention to genes associated with upper genital tract complications. This innovative state-of-the-art tool is expected to advance human gene discovery and identify novel genes linked to the susceptibility to and severity of *C. trachomatis* disease.

Further research on the effects of common SNPs in PRR receptors in the risk of tubal damage and TFI as a result of uncleared *C. trachomatis* infection may be a step towards successful advances in personalised medicine. Improving diagnostic protocols for subfertility by enabling risk group stratification of women at the highest risk of complications due to persistent genital *C. trachomatis* is a promising direction for a future development, aimed at addressing important health needs.

In conclusion, the *NOD1* +32656 GG insertion variant appears to protect against the infection with *C. trachomatis*, whereas it acts as a risk factor in developing TFI in women with a past *C. trachomatis* infection. Also, the GG insertion leads to a higher occurrence of symptoms in *C. trachomatis*-positive women.

Our finding supports the involvement of NOD1 in *C. trachomatis* recognition and subsequent responses. Taking into account previously published research, it appears that

NOD1 has a vital role in bacterial sensing in humans and other species, but specific actions elicited by each of these receptors should preferably be examined for different bacterial species or groups separately. Variations in these pathogen-specific roles that might be observed in different PRR receptors should not be surprising, considering the known diversity of bacterial mechanisms of pathophysiology. NOD receptors are evidently important for recognising those pathogenic bacteria that manage to surpass the extracellular or endosomal TLR sensing, as well as obligatory intracellular bacteria, such as *C. trachomatis*. Diversity of PRR receptors enables not only a greater spectrum of sensing, but also better fine-tuning of immune responses, establishing the basis for specific, effective defence.

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CHAPTER 2

Functional polymorphisms in vitamin D metabolism genes are not associated with susceptibility to *Chlamydia trachomatis* infection

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Abstract

Chlamydia trachomatis is the most common sexually transmitted bacterium worldwide. Its often asymptomatic course of infection increases chances of transmission, but also increases risk of late complications, including tubal pathology. Genetic variations in the host immune system are known to impact the course of infections, including that of *Chlamydia*.

Recent studies have shown the impact of vitamin D on the regulation of the immune system. In a recent mouse model it was shown that Vitamin D knockout mice had a more prolonged and severe urogenital *Chlamydia* infection. This indicates a potential role for the vitamin D pathway in *Chlamydia* infections.

In this study we assessed the impact of four polymorphisms in three genes (*VDR* (rs1544410 G>A, rs2228570 C>T), *CYP27B1* (rs10877012 G>T) and *CYP2R1* (rs10741657 G>A)) on susceptibility to *Chlamydia* infections.

In our cohort of 500 *Chlamydia* positive and 1331 *Chlamydia* negative women we did not observe statistically significant differences between the genotype distributions of the four polymorphisms. This suggests that *VDR, CYP27B1,* and *CYP2R1* do not play a role in susceptibility to *Chlamydia* infections. However, due to its pleiotropic nature in the immune system a role for the vitamin D pathway may not be excluded from the whole clinical course of *Chlamydia* infections (*e.g.* late complications), and further research is therefore required.

Introduction

Chlamydia trachomatis (CT) infects approximately 100 million people each year and is therefore the most prevalent bacterial sexually transmitted infection in the world (WHO, 2012). The infection is often asymptomatic and frequently remains untreated due to late diagnosis, thereby resulting in late complications, such as ectopic pregnancy and tubal factor infertility (TFI) (Mabey, 2014). The clinical course of a CT infection varies greatly between individuals, and is in part mediated by diverse host immunogenetic factors (Wang *et al.,* 2005; den Hartog *et al.,* 2006; Bailey *et al.,* 2009; Morré *et al.,* 2009; Jiang *et al.,* 2012; Al-Kuhlani *et al.,* 2014). These factors appear at this point to be the most promising biological indicators of complicated infection with *Chlamydiae*. (Ouburg *et al.,* 2009; Lal *et al.,* 2013; Malogajski *et al.,* 2013; Branković *et al.,* 2014).

Investigations of the role of the active form of vitamin D ($1,25\text{-}(OH)_2D_3$) within different types of cells of the immune system attest to its immunomodulatory properties (Stoffels *et al.,* 2006; White *et al.,* 2008; Hewison & Litwack, 2011; Hewison, 2012). Aside from its well-established roles in calcium homeostasis and regulation of bone density, interactions of vitamin D with the immune system have been detected several decades ago. However, it was only in recent years that the precise nature of these interactions became clearer (Holick, 2007; Hewison & Litwack, 2011). All types of immune cells can respond to the stimulation by $1,25\text{-}(OH)_2D_3$. In monocytes and macrophages the synthesis of vitamin D active form from its circulating form by 25-hydroxyvitamin-D-1α-hydroxylase (also referred to as 1-OHase, encoded by *CYP27B1*) is regulated via mechanisms entirely independent of calcium metabolism (Stoffels *et al.,* 2006). In that respect, we can distinguish two distinct groups of functions of vitamin D: one as a circulating hormone with skeletal homeostasis as its primary role; and the other one akin to a cytokine with localised production and activity (Adams & Hewison, 2010). A growing body of studies from recent years has been published on the nature of its functions as the regulator of the immune system in responses to infections. Exposure to *Mycobacterium tuberculosis* affects local expression of genes within vitamin D biopathway (Liu *et al.,* 2006; Liu *et al.,* 2008). The initial event involves sensing of specific bacterial components by pattern-recognition-receptors (PRRs), such as Toll-like receptors (TLRs). This event triggers changes in the local vitamin D metabolism in these cells, upregulating the expression of the aforementioned 1-OHase, but also of vitamin D receptor (VDR) (Liu *et al.,* 2006; Liu *et al.,* 2008; Holick, 2010). Specifically, the activation of the TLR2/1 heterodimer leads to the induction of 1-OHase and VDR, which in turn upregulate antimicrobial peptides cathelicidins (Liu *et al.,* 2006). It has been shown that VDR, upon activation by $1,25\text{-}(OH)_2D_3$, acts as a transcription factor for genes for cathelicidins and other host defence peptides, such as defensins (Wang *et al.,* 2004). Moreover, the role of the active form of vitamin D in regulating the expression of cathelicidin is not restricted to monocytes and

macrophages, but also epithelial cells on 'barrier surfaces' such as mucosa, skin and the gut (Liu *et al.,* 2008). Treating cell cultures with vitamin D's active form leads to upregulation of cathelicidins and defensins in response to *Pseudomonas aeruginosa* and *Escherichia coli* (Pier, 2000; Wang *et al.,* 2004). These peptides were also demonstrably bactericidal when applied *in vitro* to CT (Yasin *et al.,* 1996a; Yasin *et al.,* 1996b; Ramanathan *et al.,* 2002; Donati *et al.,* 2005), hence we assume that depletion of antimicrobial peptides in response to insufficient levels of vitamin D's active form could potentially lead to less effective eradication of this pathogen. Other notable functions of vitamin D in antimicrobial responses involve promotion of autophagy. He and colleagues (2013) observed in their murine model study that genital chlamydial infection was more severe and prolonged in $VDR^{-/-}$ knock-out mice compared to $VDR^{+/+}$ mice. Inflammatory response also lasted longer in the knock-out mice, which was likely due to the observed down-regulation of leukocyte elastase inhibitor (LEI), an anti-inflammatory protein.

Lack of vitamin D may therefore lead to heightened risk of developing an infection due to the compromised antibacterial actions of the immune system and barrier tissues (Hewison & Litwack, 2011). In recent years, a re-evaluation of what should be considered by vitamin D deficiency is taking place. Instead, the concept of *suboptimal* vitamin D levels is now being commonly distinguished. Suboptimal levels are not associated with deficiency-related diseases such as rickets, but nevertheless exert their negative effect on health. Therefore, it is not only deficiency, but *insufficiency* that is now considered to negatively affect the response to diseases (Hewison & Litwack, 2011). As a great proportion of the global population appears to have insufficient serum levels of vitamin D (Holick, 2007; Mithal *et al.,* 2009), this undoubtedly raises various issues from the perspective of public health and prevention. If the circulating form plasma levels are too low, as is the case in vitamin D insufficiency, 1-OHase in monocytes and macrophages lacks substrate for synthesising the active form. The outcome is insufficient levels of $1,25\text{-}(OH)_2D_3$, resulting in less binding to and activation of VDR. Hence, the VDR-regulated expression of antimicrobial peptide genes would be diminished, leading to a less effective defence against the pathogen (Hewison, 2010). The availability of the circulating form, however, may also depend on the functionality of enzymes and transport molecules constituting the vitamin D metabolic pathway. Furthermore, the conversion into the active form and the rate of reverse conversion into the circulating form can also modulate the amount and activity of vitamin D in target cells. Therefore, different polymorphisms in genes for conversion enzymes, transport molecules, and the VDR can affect host defence via the change in bioavailability of $1,25\text{-}(OH)_2D_3$ and its activation of transcription-regulating functions of the VDR (Jurutka *et al.,* 2000).

Genetic differences in genes involved in biosynthesis, serum transport, and cellular conversion of vitamin D and its precursors and metabolites have been shown to influence bioavailability of vitamin D in humans, as well as health status for various infections

(Hansdottir *et al.,* 2008; Roth *et al.,* 2008; Segaert *et al.,* 2008; Moodley *et al.,* 2013). To date, no studies examining polymorphisms within genes of the vitamin D biopathway and CT have been published. Therefore, we conduct an analysis of polymorphisms with reported functional effect on vitamin D metabolism.
We investigated whether there were differences in susceptibility to urogenital CT infection in humans, based on polymorphisms within the following genes of the vitamin D pathway: *VDR*, *CYP2R1* and *CYP27B1*. Their function is briefly outlined in Table 1.

Gene	*Protein*	*Function*
CYP2R1	vitamin D 25-hydroxylase	Converts the pre-vitamin D (synthesised in the skin) into its main circulating form, 25-OHD_3, in the liver.
CYP27B1	25-hydroxyvitamin D_3 1-alpha hydroxylase (1-OHase)	Catalyses the hydroxylation of 25-OHD_3 (circulating form) to 1,25-$(OH)_2D_3$ (bioactive form of Vitamin D)
VDR	vitamin D receptor	Upon activation by vitamin D active form, binds to retinoid-X receptor (RXR-α) and acts as a transcription factor for vitamin D response element genes

Table 1. *Genes in the vitamin D biopathway researched in this study.*

Methods

Study population

In the study, we used samples from three cohorts obtained from three Dutch STD outpatient clinics: in Amsterdam, South Limburg, and The Hague. We selected Dutch Caucasian women for this study and all participating women were between 18-33 years of age. All women were asked to sign an informed consent. *Chlamydia* status was assessed by the outpatient clinics using the available NAAT assays at the respective clinics. The Medical Research Involving Human Subjects Act (WMO, Dutch Law) stated that official approval of the study by the Medical Ethical Committee does not apply to our anonymous human material collected (MEC Letter reference: # 10.17.0046). The local medical ethical committee also approved this study, based on the fact that in the Netherlands ethical approval is not required for a retrospective use of de-identified clinical samples. Nevertheless, since we performed host genetic marker studies in relation to Chlamydial infection, we made sure all participants signed informed consent forms.
After performing appropriate χ^2 testing, we merged the three cohorts. In total, we collected 1831 samples (500 cases and 1331 controls). Upon genotyping, data were

successfully retrieved for 77-79% of case samples and 76 -82% control samples, depending on the polymorphism (Figure 1).

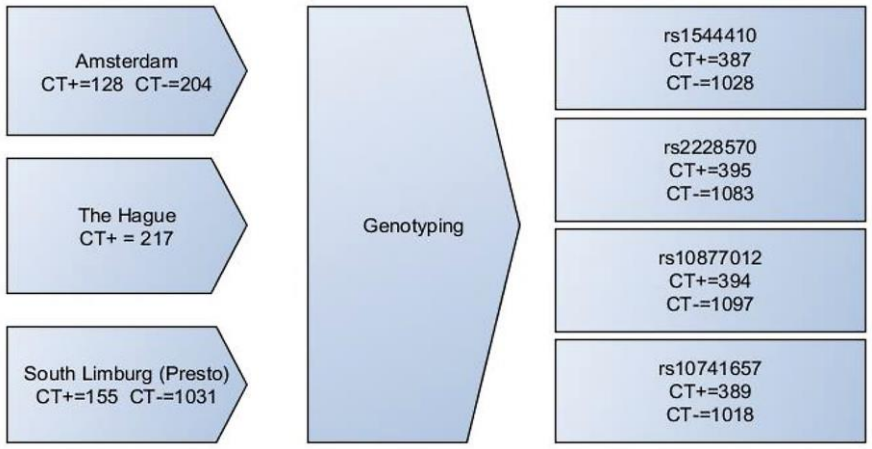

Figure 1. *Composition of the cohorts used in the study and data available after genotyping.*

Selection of polymorphisms and laboratory analyses

We selected polymorphisms for analysis based on the literature featured in Genetic Associations database, as well as PubMed searches using terms "vitamin D", "polymorphism OR SNP", and "infection". Only polymorphisms with minor allele frequencies higher than 5% were considered (based on the data in NCBI SNP database, dbSNP). We included the SNPs from the following genes: *VDR* (rs1544410 G>A, rs2228570 C>T), *CYP27B1* (rs10877012 G>T) and *CYP2R1* (rs10741657 G>A). All polymorphisms were genotyped using Roche LightCycler 480 Real-Time PCR under standard conditions.

Analyses

Groups were tested for Hardy Weinberg Equilibrium to assess Mendelian Inheritance. Fisher Exact and χ^2 tests were used where appropriate. A p-value <0.05 was considered statistically significant.

Results

The genotype frequencies of the four studied SNPs were in Hardy–Weinberg equilibrium. Genotype frequencies were uniformly distributed across the different cohorts ($p > 0.05$; data not shown) and therefore could be combined into one large study group. Differences in genotype distribution between CT positive and negative women – as assessed by the presence / absence of CT DNA detected by real-time PCR – were tested with Fisher Exact and/or χ^2 tests, and are presented in table 2.

Gene	*SNPs*	*MAF*	*Genotype*	*N(controls)*	*N(cases)*	*P*	*OR*	*95% CI*
VDR	rs1544410	0.438	GG	393 (38.2%)	134 (34.6%)			
	(G>A)		GA	477 (46.4%)	199 (51.4%)			
			AA	158 (15.4%)	54 (14.0%)			
			*A/GG			0.21	1.17	0.92-1.49
			AA/G*			0.55	0.89	0.64-1.25
VDR	rs2228570	0.412	CC	426 (39.4%)	170 (43.0%)			
	(C>T)		CT	491 (45.3%)	177 (44.8%)			
			TT	166 (15.3%)	48 (12.2%)			
			*T/CC			0.21	0.86	0.68-1.08
			TT/C*			0.13	0.76	0.54-1.08
CYP27B1	rs10877012	0.336	GG	518 (47.2%)	195 (49.5%)			
	(G>T)		GT	456 (41.6%)	165 (41.9%			
			TT	123 (11.2%)	34 (8.6%)			
			*T/GG			0.44	0.91	0.73-1.15
			TT/G*			0.18	0.75	0.50-1.11
CYP2R1	rs10741657	0.333	GG	432 (42.4%)	165 (42.4%)			
	(G>A)		GA	437 (43.0%)	173 (44.5%)			
			AA	149 (14.6%)	51 (13.1%)			
			*A/GG			1.00	1.01	0.79-1.27
			AA/G*			0.49	0.88	0.63-1.24

Table 2. *Overview of analysed polymorphisms with genotype frequencies for controls and cases, and results of their associations with susceptibility to CT infection (95% CI = confidence interval MAF = minor allele frequency; OR = odds ratio; P = p values for genotype frequency distribution).*

We did not observe statistically significant differences in genotype distributions of these four SNPs between CT positive and negative women.

Discussion

We report on our case-control study in which we assessed potential associations between four functional polymorphisms in the vitamin D biopathway genes (*VDR* (rs1544410, rs2228570), *CYP27B1* (rs10877012) and *CYP2R1* (rs10741657)) and susceptibility to urogenital CT infection. We did not detect any statistically significant associations of these polymorphisms with the infection.

VDR encodes the intracellular vitamin D receptor which is involved in the regulation of transcription for a number of genes and can form dimers with RXR-α, including those genes whose products are involved in antimicrobial defense. The effects of mutant allele of rs1544410 may thus be modulated by particular variations in the gene encoding the RXR-α protein, given their potential for interaction and gene expression alterations. Successive studies should take into account potential interactions between polymorphisms in these two genes and investigate the nature of the interaction.

There is a near-trend for rs2228570 when comparing carriage of the minor allele to the major allele homozygotes [OR=0.13]. The near-trend value is however lost when taking multiple testing into consideration. The result might suggest a lack of statistical power. Moreover, a trait analysis might reveal a combined effect of rs2228570 with other polymorphisms with functional consequence on VDR. Nevertheless, it is likely that if an actual reduction in risk indeed exists, it would be relatively small, based on the lower limit of the confidence interval. Polymorphism rs2228570, also referred to as *Fok*I, or the start codon polymorphism or SCP, was later on defined using the *Fok*I restriction enzyme in an RFLP test (Arai *et al.,* 1997). Thus, two protein variants can exist corresponding to the two available start sites: a long version of the VDR protein is the T-allele (minor allele) or the "f" allele; and also referred to as the M1 form, i.e., the methionine at first position). A protein variant shortened by three amino acids is the C-allele (major) detected as the "F" allele; also referred to as the M4 form, i.e., the methionine at fourth position) (Uitterlinden *et al.,* 2004). The direction of our odds ratio for rs2228570 would suggest a mildly protective effect of its minor allele, but that is not concordant with the findings of other studies (Colin *et al.,* 2000; van Etten *et al.,* 2007). In human monocytes and dendritic cells with a homozygous short (CC) VDR genotype, expression of IL-12 (mRNA and protein) was higher than in cells with a long (TT) VDR genotype (van Etten *et al.,* 2007). This polymorphism therefore affects immune cell behavior, with a more active immune system for the short version of VDR.

Enzyme coded by *CYP2R1* converts vitamin D into the circulating form, thereby enabling its transport to target cells where they can be converted into physiologically active (hormonal) form.

Vitamin D polymorphisms and other diseases

Our researched four polymorphisms have so far been investigated in regards to susceptibility for a small number of other infectious diseases, namely tuberculosis, acute lower respiratory infection (ALRI), and chronic hepatitis C (HCV) (Wilkinson *et al.,* 2000; Roth *et al.,* 2008; Lange *et al.,* 2011). *VDR* polymorphism rs2228570 was particularly researched. There is more evidence for their roles in non-communicable diseases, such as cancer, diabetes and autoimmune disorders (Simmons *et al.,* 2000; Lee *et al.,* 2011; Lee *et al.,* 2012; Li *et al.,* 2014). These susceptibilities were in cases of some diaseases ethnicity-specific (Lee *et al.,* 2011).

The rs2228570 TT genotype was associated with the risk of ALRI that was approximately seven times that of the CC genotype (Roth *et al.,* 2008). In the study of Wilkinson *et al.,* (2000), when tuberculosis patients were stratified according to localised or severe disease, there was a significant excess of patients with anatomically localised (extrapulmonary) tuberculosis bearing the T allele. Therefore, it appears that the minor (T, "f") that confers the longer protein variant of VDR does lead to a heightened risk of these infections.

Stimulation with bioactive vitamin D leads to decreased expression of MHC class II molecules and co-stimulators in B cells, as well as to decreased proliferation, differentiation, and cytokine production and immune response in T cells (Gorman *et al.,* 2007; Smolders *et al.,* 2008). A theory is that the production of *CYP27B1*, as well as activation of vitamin D by T cells and dendritic cells in extralymphoid tissues, might increase the T-cell programming of antigen-specific response, the so-called T-cell homing (van Etten *et al.,* 2008). Overall, the findings support a suppressive role of vitamin D in the adaptive immune system (Sundqvist *et al.,* 2010).

Low levels of vitamin D in serum are associated with a higher risk of developing MS (Munger *et al.,* 2006), and several studies show that MS patients have lower levels of 25-OHD_3 compared to controls (Smolders *et al.,* 2008). A recent study showed that high circulating 25-OHD_3 levels among MS patients were associated with improved regulatory T-cell function. It also had an impact on T-helper (Th) cells, shifting the balance in favour of Th2 cells (Smolders *et al.,* 2009).

Currently, the *CYP27B1* rs10877012 promoter polymorphism is regarded to be more important than polymorphisms within VDR genes at least in the development of endocrine autoimmune disorders. Additionally, in chronic hepatitis C (HCV) patients with genotype AA of the 1-OHase promoter polymorphism, higher serum concentrations of 1,25-$(OH)_2D_3$ were observed compared to patients with genotype AC or CC (Lange *et al.,* 2011). This was

unexpected since other studies suggested that the CC genotype constitutes a pathogenic co-factor in immune dysfunction by impairing intracellular 1,25-$(OH)_2D_3$ levels in mononuclear cells and T cells (Lopez *et al.,* 2004; Bailey *et al.,* 2007). The adverse genotype CC was more frequent in patients with chronic hepatitis C compared to healthy populations, which may be a hint that resolution of acute hepatitis C is supported by the beneficial genotype AA. 1,25-$(OH)_2D_3$ is the bioactive and, therefore, relevant form of vitamin D, which is a key player in innate and adaptive immunity. Active vitamin D has the potency to significantly enhance T cell receptor signalling, which might play an important role in establishing a sufficient adaptive immune response during acute hepatitis C or to overcome T cell exhaustion during chronic hepatitis C (van Etten *et al.,* 2007).

It is possible that vitamin D-induced antibacterial peptides are not crucial for susceptibility to CT infection in humans the same way they appear to be in cases of several other diseases. Although their bactericidal capacity was demonstrated in several *in vitro* studies of CT infections (Yasin *et al.,* 1996a; Yasin *et al.,* 1996b; Ramanathan *et al.,* 2002; Donati *et al.,* 2005), more studies are required to elucidate whether these peptides are effective against CT within host cells. Their effect might also vary depending on the stage of the chlamydial biological cycle.

Aside from upregulating antibacterial peptides upon successful PRR sensing, vitamin D is required for IFN-γ-mediated antimicrobial functions in macrophages (Fabri *et al.,* 2011). By releasing IFN-γ, T cells enhance microbicidal capabilities of macrophages, stimulating antimicrobial peptide production, endolysosomal killing and autophagy. This effect is achieved through IL-15 induction by IFN-γ, which is also capable of up-regulating *VDR* and *CYP27B1* (Fabri *et al.,* 2011). In that respect, vitamin D is a mediator of both the innate and adaptive immune response to pathogens. IFN-γ is an important cytokine in specific responses to CT and the clearance of the infection (Gottlieb *et al.,* 2010). It would be recommended for that reason to additionally examine polymorphisms in *IFNG* gene and perform trait analyses.

In conclusion, our case-control study did not register any associations between functional polymorphisms in the vitamin D biocascade and susceptibility to *Chlamydia* infections. This does not exclude a potential role for the vitamin D pathway in the severity of *Chlamydia* infections (*e.g.* development of tubal pathology). Other studies point to a more complex relationship between circulating levels of vitamin D, its intracellular conversion rate into the active form, expression of vitamin D response genes, and their regulation of the innate and adaptive immunity. CT infection requires the involvement of different immune mechanisms, and vitamin D regulates these mechanisms in specific manners. It would be important to analyse how its effect on the adaptive immunity (especially IFN-γ-associated Th1 response) versus innate mechanisms is achieved and how it relates to urogenital CT infection. Further research is needed to elucidate these processes and explain how these actions exert their effect and whether there are disease-specific

properties differ from their previously researched pleiotropic involvements in inflammatory conditions underlying for instance cancer, tissue injury, and atherosclerosis.

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CHAPTER 3

Profiling HPV infection and cervical cancer: a comprehensive overview of investigated immunogenetic polymorphisms

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Abstract

We provide an overview of all data available on the immunogenetic factors of human papillomavirus (HPV) infections, cervical intraepithelial neoplasia (CIN), and cervical cancer, published up to August 2014. We retrieved the studies for our research by performing searches on PubMed and HuGE navigator. Studies on HLA (MHC) and KIR genes were eventually excluded, due to the large amount of studies on these genes. The results are divided into sections, based on which function the product of the gene performs: cytokines (pro-inflammatory and anti-inflammatory), chemokines, receptors, and other genes involved in immune response and mediation. We end the review with concluding remarks on the implications of aggregated knowledge, call for additional, research and future perspectives.

Introduction

Epidemiology of HPV and cervical cancer

Infection with human papillomavirus (HPV) is very common across human populations. Worldwide, prevalence estimates of HPV infection among women range from 2% to 44% (Baseman & Koutsky, 2005). HPV infection may lead to cervical cancer and invasive cervical cancer is one of the most common malignant diseases among women, representing almost 10 per cent of all cancers in the female population. Each year more than 500.000 women are diagnosed with cervical cancer, mostly in developing countries (Torpy *et al.,* 2007).

Approximately 130 HPV types have been identified to date, with new types being constantly discovered. Types may differ in tissue tropism and may preferentially infect skin or mucosa. Some HPV types cause genital, oral, and throat warts, or cervical, penile, vulvar, or vaginal cancer, while others cause no symptoms, diseases, or neoplastic changes (Al-Daraji & Smith, 2009). Types 16 and 18 cause about 60-80% of cervical cancer cases. Together with type 31 and 45, they are the prime risk factors for cervical cancer (Bosch *et al.,* 2007).

As is known today, infection with high-risk human papilloma viruses (or hr-HPVs) is necessary for the development of cervical cancer, although HPV infections alone are not sufficient to cause lesions. Secondly, numerous women contract an oncogenic HPV at some point in their lives, but most of them *do not* develop cervical tumours. Thus, an effective immune response to HPV infection is believed to be an important determinant of progression and disease outcome. Transient HPV infections and regression of cervical intraepithelial neoplasia (CIN) lesions to normal epithelia suggest that immunological and genetic co-factors are involved in cervical carcinogenesis (Kanodia, Fahey, & Kast, 2007). Also, occurrence of HPV-associated cervical dysplasia in immunosuppressed patients supports the hypothesis that immune responses against persistent HPV infections play a key role in transformation of epithelial cells (Bosch *et al.,* 2007).

Host genomics and HPV

Studies have provided a wealth of information on the role of host genetics in disease aetiology and pathogenesis. It has become clear that genes encoding for immune mediators have a prominent role in susceptibility to infections and diseases, but also in severity and disease outcome. Polymorphisms in these genes may exert a protective or deleterious role on susceptibility to HPV, as well as progression to higher CIN stages and/or invasive cancer of the cervix. Although genetic technology and approaches have advanced in recent years, the full understanding of the exact role of immunogenetic genes

and their variants has still not been reached. Therefore, the key question remains: which immunogenetic polymorphisms are responsible for the striking heterogeneity in immune response against HPV between individuals?

Aims of the study

In this review we will attempt to summarise the data provided in the studies of immunogenetic factors in relation to susceptibility to different types of HPV, CIN stages, and invasive cervical carcinoma in women infected with different HPV types. We outline the most compelling data based on the included studies. Finally, we also provide a framework for future research. A detailed overview will be given per gene, subdivided per polymorphism.

Methods

We retrieved original studies on polymorphisms in genes involved in immune response and its mediation by performing searches on PubMed and HuGE navigator. The last searches were performed on August 15th 2014. Publications on major histocompatibility complex (MHC/HLA) and killer cell immunoglobulin-like receptor (KIR) genes were excluded from the review, due to a great number of studies published on the topic. There is a plethora of evidence for the effect of these genes, especially HLA, in HPV infection and progression to cervical cancer. Therefore, the results of these studies warrant a separate publication as a platform for investigating their interactions with HPV in depth.

Results

Overview of all included genes and a brief description of the functions of their products is given in table 1. In this section we will briefly summarise some of the most relevant data from the studies. Full results from all the included studies are given in tables 2-4. The results are divided into sections, based on which function the product of the gene performs. Table 2 comprises genes coding for cytokines and chemokines; table 3 presents genes coding for receptors; and in table 4 all other genes involved in immune response and mediation are given. Tables 2, 3 and 4 provide the following information in columns: gene; polymorphism; cohort (pathology); number of included cases; ethnicity of the population; HPV type (if known); genotype frequencies in the control population; study results; and the study reference.

Table 1. *Genes reported on in the review with a brief summary of their function.*

Gene/protein	*Function of the protein*
	Pro-inflammatory cytokines
IL1A	mediator of inflammation, fever and sepsis, produced predominantly by macrophages and neutrophils
IL1B	mediator of the inflammatory response, cell proliferation, differentiation, and apoptosis
IL6	stimulates production of neutrophils in the bone marrow, differentiation of B cells and has antagonistic effect on regulatory T cells
IL8	induces chemotaxis (primarily of neutrophils) and phagocytosis and is an important mediator of angiogenesis
IL12A, IL12B	two subunits that form IL12; stimulates interferon (IFN)-γ and TNF production, and is important for the differentiation and function of T cells and the activity of natural killer (NK) cells; also nti-angiogenic
IL18	works synergistically with IL12, stimulates IFN-γ production and the activity of NK and T cells
TNF/TNFA	secreted by monocytes and macrophages; implicated in tumour regression (potentially pro-apoptotic), is involved in cachexia and septic shock and can induce cell proliferation and differentiation; can cause fever directly or indirectly via stimulation of IL-1 production
LTA	mediates a variety of inflammatory, immunostimulatory, and antiviral responses, is involved in the formation of secondary lymphoid organs during development; also plays a role in apoptosis
IFNG	produced predominantly by NK cells and NKT cells, but also by Th1 cells and CTLs; most important function is the direct inhibition of viral replication, but it can also play a role in bacterioses
IFNA17	Function not fully elucidated
	Anti-inflammatory cytokines
IL1RN	natural inhibitor of the pro-inflammatory effect of IL1β
IL10	inhibits the function of NK cells during the immune response to viral infections. This cytokine shows both immunosuppressive and anti-angiogenic effects, therefore it can paradoxically act in tumour promoting as well as tumour inhibiting fashion
TGFB	controls cellular proliferation and differentiation; stimulates differentiation of regulatory T cells and Th17; can block the function of lymphocytes and monocyte-derived phagocytes
	Chemokines
CXCL12 (SDF-1)	strongly lymphocytic chemoattractant; promotes angiogenesis, involved in carcinogenesis and tumour progression
	Receptors
CCR2	receptor for CCL2; mediates monocyte chemotaxis; aids inflammatory response against tumours
CCR5 (CD195)	mediates T cell chemotaxis; implicated in several diseases, *i.e.* HIV and West Nile virus
CD28	receptor for CD80 and CD86; expressed on T cells as a co-receptor; provides co-stimulation that acts additionally to T cell receptor (TCR) stimulation
CD32 (FCGR2A)	one of B cell co-receptors; provides a feedback mechanism that down-regulates excessive IgG production
CD80	on B cells and monocytes; delivers co-stimulatory signal to T cells via its interaction with CD28
CD83	expressed mainly by dendritic cells (DC); mediates stimulation of newly primed T cells
CD86	on antigen-presenting cells (APCs); delivers co-stimulatory signal to T cells via its

Gene/protein	*Function of the protein*
	interaction with CD28
CD278 (ICOS)	present on activated T cells; possible role in Th2 cell differentiation and activity
CD279 (PDCD1)	a T cell-regulating protein; appears to be involved in the down-regulation of T-cell responses and anti-tumour responses
CTLA4	dominant down-regulating receptor in the T-cell-mediated anti-tumour responses; blocking CTL4 leads to more efficient immune response to tumours
FAS (TNF6SF6)	a death receptor on the cell surface that upon ligand binding leads to apoptosis of the cell
FASL	binds to FAS and induces apoptosis; interactions between FAS receptor and FASL are important in the regulation of the immune system cancer progression
IFNGR2	non-ligand-binding beta chain of the IFN-γ receptor
IL4RA	codes for alpha chain of IL4 receptor; can bind IL4 and IL13 and regulate IgE antibody production; can promote differentiation of Th2 cells; soluble form can inhibit IL4-mediated cell proliferation and IL5 up-regulation
IL8RA, IL8RB	encodes subunits of the IL8 receptor
IL10RA	encodes IL10 receptor
MBL1, MBL2	recognises carbohydrate residues on the surface of bacteria, viruses, protozoa and fungi; recognition activates the lectin pathway of the complement system
NGK2C	
TLR2	pattern recognition receptor that senses the presence of bacterial lipoproteins and other components of bacteria and fungi; evidence suggests role in also sensing viruses
TLR4	recognises lipopolysaccharide molecules present in Gram-negative bacteria; evidence also points to a role in sensing viruses
TLR9	recognises un-methylated CpG DNA sequences ubiquitous in bacterial and viral genomes; anchored in intracellular membranes (endosomes, lysosomes); HPV oncoproteins E6 and E7 block its expression
	Other
CYBA	Cytochrome b-245 light chain, component of the superoxide-generating microbicidal complex present in phagocytes
ERAP1 (ARTS-1)	located in the endoplasmic reticulum; function in protein processing and transport
FANCA	involved in post-replication DNA repair; helps maintain chromosomal stability in hematopoetic cell line; mutations in the gene often lead to anemias and cancers
IRF1	transcriptional regulator, stimulates immune response, regulates apoptosis, DNA repair and enhances tumour suppression
IRF3	role in regulating interferons that control viral infections; also regulates interferon-inducible genes
JAK3	expressed mainly in immune cells, where it transduces signals in response to activation by interleukin receptors
NFKB1	transcription factor, activated by stimuli such as cytokines, free radicals, and recognition of bacterial or viral structures by PRRs; regulates transcription of a large number of genes, necessary for a proper immune response and inflammation
OAS1,OAS2,OAS3	enzymes that activate latent ribonuclease L (RNAse L), resulting in viral RNA degradation and inhibition of protein synthesis
PSMB8 (LMP7), PSMB9 (LMP2)	catalytic subunits of the immunoproteasome, important for processing class I MHC peptides; inducible by IFN-γ
SULF1	Golgi enzyme involved in anti-proliferative and pro-apoptotic responses to exogenous stimulation; may be down-regulated in certain cancers
RNASEL	RNAse L plays an antiviral role in the cell, but can additionally be triggered by interferons to degrade RNA molecules as part of the pro-apoptotic response to stress or threat
SLC11A1	transmembrane transporter of divalent ions, regulates cell's ion levels and can confer resistance to certain pathogens
SMAD7	cell signaling molecule, acts as a TGFβ1 receptor antagonist; appears to be involved in

Gene/protein	Function of the protein
	tumourigenesis, but the exact role is still unclear
TAP1,TAP2	involved in antigen presentation, by assisting the transportation of degraded peptides into the endoplasmic reticulum for assembly with MHC molecules
TMC6 (EVER1), TCM8 (EVER2)	transporters in the endoplasmic reticulum; mutations associated with epidermoplasia verruciformis, or abnormal susceptibility to HPV
TNFAIP8, TNFAIPL1	pro-oncogenic and anti-apoptotic signaling molecules

Discussion

In this section, we will highlight the findings on extensively researched genes and their respective polymorphisms. These findings illustrate the impact of polymorphisms in genes for cytokines, chemokines, immune receptors in HPV infection, persistence, and progression into cervical neoplastic stages. The complete overview of all obtained findings in given below at the end of the chapter, in tables 2-4.

Cytokines

Polymorphisms in genes coding for secreted polypeptide regulators of the immune system play a major role in HPV persistence and progression of CIN stages and to cervical cancer.

Pro-inflammatory cytokines

Cytokines that underlie inflammatory processes are typically produced in response to a presence of pathogens and tumour antigens, but they can also be induced by up-regulated levels of other pro-inflammatory cytokines.
Evidence was found for the involvement of several gene polymorphisms for *IL1A, IL1B, IL6, IL8, IL12A, IFNG, INFA17, TNF* and *LTA* in progression of CIN stages, progression to cervical cancer and viral persistence (table 2). Several studies consistently point to the effect of *IL1B* -511 C>T in increasing risk of cervical cancer in Korean, North Indian, Chinese Han, and Egyptian populations (Kang *et al.,* 2007; Singh *et al.,* 2008; Qian *et al.,* 2010; Al-Tahhan *et al.,* 2011).
Variation in the *TNF* gene is one of the most researched in relation to HPV and cervical cancer. Polymorphism *TNF* -308 G>A was especially studied extensively. In a British cohort, all categories of CIN were associated with *TNF* -308 low secretor phenotype GG, while in Indo-Aryan and Portuguese women the A allele was associated with a three-times increase in susceptibility to HPV16 and a twofold increased risk of developing cervical cancer in Portuguese women (Ghaderi *et al.,* 2000; Jang *et al.,* 2001; Stanczuk *et al.,* 2003; Ivansson *et al.,* 2010). Also, conflicting results were found in other polymorphisms, such as

TNFa microsatellite polymorphism. In a Swedish Cervical cancer cohort, *TNFa*-11 was associated with susceptibility to HPV18 infection, not HPV16 infection (Ghaderi *et al.,* 2001). Whereas in another study on patients with CIN, the *TNFa*-11 allele was significantly more frequent in HPV16 seropositive patients compared with sero-negative patients, while no association was found with HPV18 infection (Ghaderi *et al.,* 2000). However, different observations might be due to small population sizes (in the case of the TNFa microsatellite studies) or not determining the presence of particular HPV types in relation to the carcinoma (several studies on *TNF* -308 G>A).

IFN-γ represents one of the most potent antiviral cytokines. *IFNG* +874 T>A (rs2430561) is its most researched polymorphism in respect to HPV infection and cervical neoplasia. The AT and AA genotypes were associated with increased risk for cervical cancer in a North Indian cohort (Kordi *et al.,* 2008). In another North Indian study, the AA genotype was associated with higher risk of cervical cancer, higher disease stage and in smokers also with susceptibility to cervical cancer (Gangwar *et al.,* 2009). The AA genotype was also associated with increased susceptibility to cervical cancer in Chinese (Wang *et al.,* 2011). An explanation could be that carriers of the AA genotype have lower IFN-γ production which might lead to a less effective antiviral response. However, in Brazilian and Swedish studies no significant differences were found (Fernandes *et al.,* 2008; Guzman *et al.,* 2008; Ivansson *et al.,* 2010). Genotype combination *CTLA4*-319(CC)/*IFNG*+874(AA) was associated with *reduced* cervical cancer susceptibility (Ivansson *et al.,* 2010) in a Swedish study. It is not immediately clear how this may be mediated. *CTLA4*-319C>T has not been found to be associated with any cervical changes in that same study.

Anti-inflammatory cytokines

Variants responsible for down-regulating inflammation seem to have an important effect on HPV infection and cervical neoplasia.

The *IL1RN* 86 bp VNTR 4/2-repeats genotype was found to be protective against HPV16 and HPV18, and the 5/2- and 5/4-repeats genotypes were associated with a higher risk of cervical cancer in North Indians (Kordi Tamandani *et al.,* 2007). In contrast, the frequency of *IL-1RN* 2/2- and 4/2-repeats genotypes was significantly higher in cases than controls in another study in India (Singh *et al.,* 2008). In Austrian Caucasians with CIN 2/3 lesions no significant difference was found for that variant (Grimm *et al.,* 2011). In haplotype analysis the 1T (*IL1RN**4repeats/*IL1B* -511*T) and 2T (*IL1RN**2repeats/*IL1B* -511*T) were associated with the risk of cancer in the North Indian population (Singh *et al.,* 2008). Clearly, the physiological activity of IL1Ra considerably depends on genetic variation; therefore it should be studied more extensively as well as in other, non-Asian populations.

IL-10 shows both immunosuppressive and anti-angiogenic effects, therefore it can paradoxically act in tumour promoting as well as tumour inhibiting fashion. Associations

with both increased and decreased IL10 levels in cervical cancer have been determined in different studies (Wang *et al.,* 2013). *IL10* -1082 G > A influences the IL10 production. The GG genotype is associated with high production, AG with intermediate production and AA with low production. This SNP was investigated in several populations with conflicting results; British carriers of at least one G-allele without cervical lesions were more likely to clear HPV infections (Farzaneh *et al.,* 2006), while in Zimbabwe and Japan, the G allele was associated with increased disease severity (Stanczuk *et al.,* 2001; Matsumoto *et al.,* 2010) . In Korean, Chinese, Dutch and Hungarian cohorts -1082 did not reach statistical significance (Roh *et al.,* 2002; Szoke *et al.,* 2004; Zoodsma *et al.,* 2005; Wang *et al.,* 2010). A recent meta-analysis concluded that this polymorphism most likely has no effect on cervical cancer risk (Ni *et al.,* 2013). One study, however, established a connection between -1082 polymorphism and oral contraceptives. Their use led to an increased susceptibility to, and risk of development and progression of cervical lesions in HPV-positive Brazilian women (Chagas *et al.,* 2013). -819 C > T was not significant in Korean (Roh *et al.,* 2002), Mexican (Torres-Poveda *et al.,* 2012) and Brazilian populations (Chagas *et al.,* 2013). -592 C > A (rs1800872) was not significant in Swedish and Korean cohorts but in the Dutch, heterozygotes were more likely to have CIN II/III lesions or SCC and in Mexico, carriers of the A allele had a two-fold increased chance of developing cervical intraepithelial lesions (Roh *et al.,* 2002; Zoodsma *et al.,* 2005; Ivansson *et al.,* 2007; Torres-Poveda *et al.,* 2012). In Asian populations in particular, -592 C > A was found to be associated with developing cervical cancer(Ni *et al.,* 2013). Haplotypes consisting of -1082/-819/-592 were investigated in the Dutch, Brazilians, Mexicans, and Koreans but in none of these studies differences were found (Roh *et al.,* 2002; Zoodsma *et al.,* 2005; Fernandes *et al.,* 2008; Torres-Poveda *et al.,* 2012). The paradoxical activities of IL10 on carcinogenesis can have implications on HPV infection and cervical cancer, given that angiogenesis bears importance in late stages of cancer, but not in viral infection and persistence. It is therefore biologically plausible that the heterogeneous effect of *IL10* polymorphisms in HPV and cervical cancer might be tied to the stage of lesions. Research on IL10 should be furthered to investigate these roles.

Chemokines

We retrieved only one study on chemokine polymorphisms in HPV and cervical carcinoma. *CXCL12 (SDF-1)*. Only two, rs266085 and rs266093, had any effect (table 2). The minor (A) allele of rs266085 was inversely associated with cervical cancer. The minor (G) allele of rs266093 was associated with a weakly increased risk of cervical cancer(Maley *et al.,* 2009). More research on the roles of genetic variability in chemokine genes is warranted.

Receptors

The ligands for receptor molecules can be cytokines, chemokines, co-stimulation transmembrane proteins, or pattern-recognition receptors (PRRs). We retrieved studies on polymorphisms in the following genes in relation to HPV infection and cervical neoplasia *CCR2, CCR5 (CD195), CD28, CD32 (FCGR2A), CD80, CD83, CD86, CD278 (ICOS), CD279 (PDCD1), CTLA4, FAS (TNF6SF6), FASL, IFNGR2, IL4RA, IL8RA, IL8RB, IL10RA, MBL1, MBL2, NGK2C, TLR2, TLR4, and TLR9*. Based on the studies, we observe a varying role of different receptors in respect to these diseases. Thorough overview of these studies is presented in Table 3. Here we will discuss only the most compelling findings.

FAS is a death receptor on the cell surface that upon ligand binding leads to apoptosis of the cell. Single-nucleotide polymorphisms (SNP) that have been identified in the promoter of the FAS gene are -1377 G>A and -670 G>A. The -1377A allele and the -670G allele disrupt Sp1 and STAT1 transcription factor binding sites respectively, and thus diminish promoter activity and decrease FAS gene expression. These two functional promoter polymorphisms are associated with increased risk of acute myeloid leukemia and systemic lupus erythematosus (Kanemitsu *et al.,* 2002; Sibley *et al.,* 2003; Lai *et al.,* 2005b). In Spanish and Chinese cohorts no associations were found for the -1377 G>A polymorphism (Lai *et al.,* 2005b; Sun *et al.,* 2005; Lerma *et al.,* 2008). -670 G>A (rs1800682) has been studied in multiple cohorts with varying results. In Chinese, Dutch and Costa Rican cohorts no significant differences were found (Sun *et al.,* 2005; Zoodsma *et al.,* 2005; Wang *et al.,* 2009). In Spain, a non-statistically-significant trend between GG genotype and adverse prognosis was observed (Lerma *et al.,* 2008). In Japan, cases with HSIL were more likely to be infected with hrHPV and have either the GA or GG genotype as compared to healthy controls and LSIL patients (Ueda *et al.,* 2005). In contrast, in Chinese Han and Taiwanese populations the A allele and AA genotype were associated with HSIL and SCC. The frequency of the A allele and AA genotype increased gradually with increased severity(from LSIL to HSIL to SCC) (Lai *et al.,* 2003; Lai *et al.,* 2005b). In Costa Rica the -252 C > T (rs1468063) and +16 G > A (rs3218619) SNPs were studied but there were no significant differences between study populations (Wang *et al.,* 2009). The *FAS* -1377A/-670A haplotype conferred a higher risk for HSIL/SCC (OR 3.05, 95% CI 1.28–7.30) than *FAS* 670A alone (OR 1.26, 95% CI 1.28–7.30) (Lai *et al.,* 2005b). A meta-analysis set out to investigate the controversial results published on *Fas* polymorphism rs180082 (Chen *et al.,* 2013). The authors conclude that there is a lack of evidence for association of this polymorphism with susceptibility to cervical cancer. They, however, point out the need for a larger scale studies that would focus on any possible gene-gene and gene-environment interactions(Chen *et al.,* 2013).

Interactions between FasR and FasL are important in the regulation of the immune system cancer progression. *FASL* -844 C > T (rs763110) is the only SNP that has been described in

combination with cervical cancer. It lies within a putative binding motif for CAAT/enhancer-binding protein (C/EBPβ) and the two resulting alleles have different affinities for C/EBPβ. The -844CC genotype may result in increased FasL expression, alteration of FasL-mediated signaling in lymphocytes, and an increase of the risk for autoimmunity(Wu *et al.,* 2003). This SNP was studied in Chinese, Chinese Han and Swedish cohorts. Only in the Chinese differences in gene expression were found between cases and controls; subjects carrying the *FASL* –844CC genotype had a threefold increased risk of developing cervical cancer (Lai *et al.,* 2005b; Sun *et al.,* 2005; Ivansson *et al.,* 2007). Haplotype analysis has been done for *FAS* -670 + FASL -844 in Chinese Han. While *FASL* -844 in itself was not significant, it did have an additive effect on the HSIL/SCC risk as compared to *FAS* -670 alone. It would be advisable to perform more studies with combinations of *FAS* and *FASL* polymorphisms to see if some of these combined variants affect the risk of cervical carcinoma.

Toll-like Receptors

Toll-Like Receptors (TLRs) are transmembrane proteins that sense pathogen-associated molecular patterns (PAMPs), the conserved structural motifs in pathogenic organisms. TLRs are normally anchored in the plasma membrane, but can also be present in intracellular membrane compartments, such as endosomes or lysosomes.

Toll-like receptor 2 (TLR2) is a pattern recognition receptor that senses the presence of bacterial lipoproteins and other components of bacteria and fungi (Shin *et al.,* 2011). However, there is mounting evidence that points to its putative role in sensing viral pathogens as well (Nischalke *et al.,* 2012; Soberman *et al.,* 2012). Polymorphism +613 T > C (rs3804099, rs3804100) showed association with decreased risk for and progression to CIN3 or cervical cancer in Costa Rican population (Wang *et al.,* 2009). A study on a North Indian population did not find the association between -196 to -174 deletion in *TLR2* and cervical cancer(Pandey *et al.,* 2009).

TLR4 plays an important role in recognizing lipopolysaccharide molecules present in Gram-negative bacteria, however, as in the case of TLR2, recognition of viruses has also been implicated (Long *et al.,* 2014). Ile/Thr genotype of the +936 C > T (rs4986791) polymorphism was associated with stage II cervical cancer in a North Indian population (Pandey *et al.,* 2009) and conferred approximately 2.5-fold risk of developing cervical cancer at an early stage, while no kind of association was not found for it in a Costa Rican study for CIN3 or cancer and persistent HPV (Wang *et al.,* 2009). Polymorphism +636 A > G (rs4986790) was investigated in the same two studies (Wang *et al.,* 2009; Long *et al.,* 2014), but relationship with CIN3, cervical cancer or persistent HPV was established.

The function of Toll-like receptor 9 (TLR9) is to recognise unmethylated CpG DNA sequences, ubiquitous in bacterial and viral genomes, for instance HPV16. Viral

oncoproteins E6 and E7 block the expression of this receptor (Hasan *et al.,* 2007). Several studies have studied the potential relationships of *TLR9* polymorphisms with HPV infection and progression to cervical neoplasia. A study by Lai and colleagues (Lai *et al.,* 2013) found that the HPV16 infection rate in Chinese Han population was 13.8 times higher in cervical cancer patients with *TLR9* +2848 (rs352140) GA/AA genotype compared to those with GG genotype. The presence of mutant allele A also contributed to the higher risk of having cervical cancer compared to the wild-type allele G. No associations were found in this study between +1486 T/C (rs187084) polymorphism and cervical cancer. The study group and the minor allele frequencies for these two SNPs were relatively small (Lai *et al.,* 2013), therefore replication studies with greater sample size would be needed. Another Chinese study investigated whether *TLR9* SNP -1486 T > C (rs187084) bears association with cervical cancer and found that the carriage of a heterozygote genotype increased the risk of cervical cancer compared to the TT genotype(Chen *et al.,* 2012). Finally, a study of the Ludwig-McGill cohort (Brazilian) failed to detect significant relationships between rs5743836 (T-1237C) SNP and cervical cancer, although it is possible that that is due to low frequency of the minor allele heterozygote (Oliveira *et al.,* 2013).

We assume that TLR9 ought to be more relevant for the recognition of HPV, as it is capable of recognising patterns in viruses as well. Therefore further research on the roles of TLR9 functional polymorphisms in HPV and cervical cancer would be recommended.

Other

In this section we discuss the most prominent findings on other genes involved in immune response and mediation that are not classified as cytokines, chemokines or receptors, in regards to HPV infection and cervical cancer. Genes reported on are *CYBA, ERAP1, (ARTS-1), FANCA, IRF1, IRF3, JAK3, NFKB1, OAS1, OAS2, OAS3, PSMB8 (LMP7), PSMB9 (LMP2), SULF1, RNASEL, SLC11A1, SMAD7, TAP1, TAP2, TMC6 (EVER1), TCM8 (EVER2), TNFAIP8,* and *TNFAIPL1*.

It appears that genetic variation in genes regulating transport, processing, and maturation of proteins and polypeptides from cytosol to endoplasmic reticulum and Golgi apparatus can affect the risk of HPV and cervical cancer. That is unsurprising, given that the virus requires these cellular compartments and their machinery to produce its own proteins, as well as human endogenous products that the virus can use for its promotion. The fate of successful antigen presentation - and thereby revealing the presence of infection to the immune system - depends on whether these gene variants affect degrading and transport of viral components and their assembly with MHC molecules. Studies show the contribution of such genes (*TAP1, TAP2, TMC8*) in susceptibility to HPV and cervical cancer. A greater number of their polymorphisms should be studied on more populations with HPV and cervical carcinoma.

Also, it would be relevant to see if *NFKB1* polymorphisms have a profound effect on HPV infection and cervical cancer risk. To our knowledge, there has been only one, and it has demonstrated the association of an insertion-deletion polymorphism -94 ATTG with squamous cell carcinoma. These additional studies would provide more insight into whether the variation in this transcription factor crucial for inflammation and immune responses to pathogens contributes significantly to differences in people's susceptibility, or whether research ought to focus more on the upstream elements of that signaling pathway.

Conclusions and indications for prospective research

There is a great diversity of immunogenetic associations in HPV infection, persistence, and cervical neoplastic transformation, based on the overwhelming body of continuously growing data. Contradicting results indicated by different studies, such as in the aforementioned case of *IL10* polymorphisms and their potential roles in risk, development and progression of cervical neoplasia, attest to the complexity of the topic. Multiple gene-gene and gene-environment interactions ought to be inspected on par, in order to gain a comprehensive, systematic insight into the molecular network underlying the etiology of HPV persistence and cervical cancer. However, as is the case with low-penetrance alleles, we can only speak in terms of attributable risks. For a considerable number of these

genes, contributions of each polymorphism or even a haplotype to a disease are often modest.

Given the great genetic diversity of HPV viruses, it is crucial to focus on particular types when investigating their potential associations with neoplastic stages. Therefore the researchers should routinely test for the types present in their tissue samples. By focusing on a particular HPV type, or a small group of genetically akin types, the roles of host genetic components are more easily identified. Also, a promising strategy would be to focus on specific signaling biopathways, for instance within the TLR-NFκB pathway, or assessing the role of polymorphisms in genes coding for particular cytokines, their receptors, signaling molecules, and transcription factors.

This review of original studies on polymorphisms in immune response genes pertinent to HPV infection and cervical cancer provides a detailed overview of the investigated associations of genes involved in immune response with HPV infection and cervical cancer, with the exception of HLA and KIR genes, which warrant a separate literature review. Due to a substantial heterogeneity (and occasional contradictory findings) of the published studies that we retrieved, their results can effectively be comparable only to a certain degree. Among the main factors contributing to this are different or incompatible study designs, the diversity of studied populations' ethnic backgrounds, presence/absence and combinations of different viral types in samples, and small number of cases in some of the studies. In order to determine whether difference in immune responses is mediated by specific HPV types, greater sample numbers are necessary. Studies that do not take genetic variation of different human groups might miss some of the effects of these polymorphisms on HPV persistence and related diseases. Furthermore, the studies on one or a small number of polymorphisms in a particular gene do not allow for a more thorough insight into potential synergistic effects of other genes or polymorphisms within various pathways or their mediating effects. Prospective studies ought to incorporate better study designs and provide more detailed information on the samples used.

The increasingly popular genome-wide association study (GWAS) approach for the purpose of identifying polymorphisms of interest appears promising for the purpose of elucidating the susceptibility to persistent HPV infection as well as progression of cervical neoplasia. Also, the emergence of integrative sciences such as systems biology and immunoinformatics appears to be a promising approach being developed that could help explain these interactions and provide a full understanding of the etiology of these diseases (Charron, 2011; Yan, 2010). Understanding the immunogenetic networks that underlie complex diseases such as cancer would hopefully bring personalised prevention, diagnosis and treatment closer to their successful implementation into the health care system. It would be interesting to see whether this can be done by incorporating these factors with other biomarkers most predictive of cervical lesions – *MAL/CADM1* methylation pattern, p16INK-4a/Ki-67 dual immunostaining and viral integration (Litjens *et*

al., 2013). These markers appear most promising for the usage in successful triage of hrHPV-positive women for the purpose of successful screening for high-grade lesions. Aside from the potential to alter diagnostic risk assessment, detailed insights into the roles of immunogenetic factors in HPV and cervical cancer can contribute to other levels of prevention as well as therapeutics. Prescribing immunomodulatory treatment based on a person's immunogenetic profile may lead to a more effective cure or remission with minimised side-effects. Developing a therapeutic HPV vaccine would provide novel means of treatment for individuals already infected with hrHPV or suffering from related diseases. Elucidating the precise role of immunogenetics in HPV infection and cervical neoplasia is a prerequisite from making these advances.

Table 2. *Overview of polymorphisms in genes coding for cytokines*

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
Pro-inflammatory cytokines								
IL1A	+12 C > T (rs1800587)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL1A	+21 G > T (rs17561)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL1A	−889 C > T	CIN 2/3	131	Austrian Caucasian		CC: 48.0 TC: 41.2 TT: 10.8	Associated with risk of CIN (p=0.01) The presence of at least one T allele was associated with a reduced risk of CIN (OR 0.3 [0.1–0.7]).	Grimm *et al* (2011)
IL1B	-1060 T > C (rs16944)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL1B	-580 C > T (rs1143627)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL1B	-511 C > T (rs16944)	SCC	182	Korean		CC: 16.8 TC: 53.3 TT: 29.9	The carriers with -511 C/T or T/T genotypes were at higher risk of cervical cancer with odds ratio of 2.42 (95% CI 1.31-4.46, p<0.005).	Kang *et al* (2007)
IL1B	-511 C > T	Cervical cancer	150	North Indian		CC: 14.81 TC: 46.91 TT: 38.27	TT genotype of IL-1b polymorphism was significantly higher in cases compared with controls (57.7 versus 38.3%; OR = 2.8; P = 0.012). TT genotype of IL-1b (OR = 5.2; P = 0.02) was associated with the higher stages (3) of cervical cancer.	Singh *et al* (2008)
IL1B	-511 C > T	CIN 2/3	131	Austrian Caucasian		CC: 44.0 TC: 48.8 TT: 7.2	NS	Grimm *et al* (2011)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL1B	-511 C > T	Cervical Cancer	404	Chinese Han		CC: 30.7 TC: 50.0 TT: 19.3	Genotypes CT/TT CC were associated with a significantly increased risk of cervical cancer (OR = 1.52; 95% CI, 1.10–2.09)	Qian *et al* (2010)
IL1B	-511 C > T	Cervical cancer	100	Egyptian		CC: 18 TC: 44 TT: 38	T/T genotype of IL-1b polymorphism was significantly higher in cervical cancer cases compared with controls (57 vs. 38%; OR = 2.16; P = 0.028) and the T allele carriage was significantly associated with cervical cancer risk (OR = 2.00, 95% CI = 1.19–3.38, and P = 0.008).	Al-Tahhan *et al* (2011)
IL1B	-31 T > C (rs1143627)	Cervical Cancer	404	Chinese Han		TT: 31.7 CT: 47.8 CC: 20.5	Genotypes TC/CC were associated with a significantly increased risk of cervical cancer (OR= 1.60; 95% CI, 1.16–2.21)	Qian *et al* (2010)
IL1B	+14 C > T (rs1143634)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL1B	+ 3953 C > T (rs1143634)	CIN 2/3	131	Austrian Caucasian		CC: 59.5 TC: 34.4 TT: 6.1	NS	Grimm *et al* (2011)
IL6	-660 A > G (rs1800797)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL6	-635 C > G (rs1800796)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL6	-236 C > G (rs1800795)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL6	-174 G > C (rs1800795)	Cervical cancer + HPV^{+}	42	Brazilian		CC: 6.9 CG/GG: 93.1	NS	Fernandes *et al* (2008)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL6	(rs2069837)	Cervical cancer	1584	Eastern Chinese	Not tested		AG/GG genotypes were found to be significantly associated with cervical cancer risk, with a statistical power of 99.1%. The carriers of these genotypes had significantly increased levels of IL6 protein expression.	Shi *et al* (2013)
IL6	(rs2069840)	Cervical cancer	1584	Eastern Chinese	Not tested		NS	Shi *et al* (2013)
IL6	-174 G > C	CIN 2/3	131	Austrian Caucasian		GG: 42.0 CG: 38.9 CC: 19.1	NS	Grimm *et al* (2011)
IL6	-174 G > C	Cervical cancer	56	Brazilian		GG: 58.5 CG: 40.3 CC: 1.2	Women carrying at least one C genotype in their IL-6 promoter region (2174GfiC) are at higher risk of developing cervical cancer.	Nogueira de Souza *et al* (2006)
IL6	Haplotypes IL1A −889/ IL1B −511/ IL1B +3953/ IL1RN VNTR/ IL6 -174	CIN 2/3	131	Austrian Caucasian		T/C/E2/long/C: 11.4 T/C/E1/long/G: 8.0 C/T/E1/2/G: 8.8 C/T/E1/long/C: 6.5 C/T/E1/long/G: 9.5 C/C/E1/2/G: 8.3 C/C/E1/long/C: 12.3 Others: 35.3	NS	Grimm *et al* (2011)
IL8	-351 A > T (rs4073)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL8	-65 C > T (rs2227538)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL12A	+277 G > A (rs568408)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL12A	rs568408	Cervical cancer	404	Chinese (Jiangsu Province)		GG: 70.5 GA: 27.7 AA: 1.7	IL12A rs568408 GA/AA variant genotypes were associated with a significantly increased risk of cervical cancer [adjusted odds ratio,1.43; 95% CI, 1.06-1.93]	Chen *et al* (2009)
IL12A	rs2243115	Cervical cancer	404	Chinese (Jiangsu Province)		TT: 83.4 TG: 15.8 GG: 0.7	NS	Chen *et al* (2009)
IL12B	-912 G > A	Invasive cervical cancer	154	Korean		AA: 5.3 GA: 30.2 GG: 64.5	GG genotype in IVS2 -912G/A SNP showed increased risk for the susceptibility to cervical cancer, as compared with AA genotype (OR 8.770; 95% CI 1.105–69.641; P = 0.040).	Han *et al* (2008)
IL12B	+314 A > C	Invasive cervical cancer	154	Korean		CC: 7.9 AC: 47.6 AA: 44.5	NS	Han *et al* (2008)
IL12B	+1188 A > C (rs3212227?)	Invasive cervical cancer	154	Korean		AA: 29.1 CA: 49.2 CC: 21.8	NS	Han *et al* (2008)
IL12B	+159 A > C (rs3212227)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL12B	Haplotypes -912 +314 +1188	Invasive cervical cancer	154	Korean			The IVS2 -912G +314A haplotype among the haplotypes between two loci was significantly associated with increased risk for cervical cancer. The -912G +314A +1188A haplotype and -912G +314A +1188C haplotype among the haplotypes between three loci were significantly associated with increased risk for cervical cancer.	Han *et al* (2008)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL12B	rs3212227	Cervical cancer	404	Chinese (Jiangsu Province)		AA: 37.1 AC: 45.8 CC: 17.1	IL12B rs3212227 AC/CC variant genotypes were associated with a significantly increased risk of cervical cancer [adjusted odds ratio, 1.30; 95% CI, 0.97-1.75]	Chen *et al* (2009)
IL12A/B	rs568408 / rs3212227	Cervical cancer	404	Chinese (Jiangsu Province)		GG – AA: 24.8 GA/AA – AA: 12.4 GG – CA/CC: 45.8 GA/AA - CA/CC: 17.1	Subjects carrying rs568408 AG/AA and rs3212227 AC/CC genotypes and having >2 parities showed a 6.00-fold (95% CI, 2.86-12.56) elevated cervical cancer risk (P for multiplicative interaction = 0.046).	Chen *et al* (2009)
IL18	-1297 T/C (rs360719)	Cervical cancer (CSCC)	470	Northern Taiwan	16, 18, other	TT: 77.8 TC: 20.8 CC: 1.4	NS	Yang *et al* (2013)
IL18	-607 C/A (rs1946518)	Cervical cancer (CSCC)	470	Northern Taiwan	16, 18, other	CC: 23.4 CA: 49.6 AA: 27.0	NS	Yang *et al* (2013)
IL18	-380 C/G (rs5744247)	Cervical cancer (CSCC)	470	Northern Taiwan	16, 18, other	CC: 37.8 CG: 44.7 GG: 17.5	NS	Yang *et al* (2013)
IL18	-137 G/C (rs187238)	Cervical cancer (CSCC)	470	Northern Taiwan	16, 18, other	GG: 77.6 GC: 21.1 CC: 1.4	NS	Yang *et al* (2013)
IL18	+105 A/C (rs549908)	Cervical cancer (CSCC)	470	Northern Taiwan	16, 18, other	AA: 77.8 AC: 20.8 CC: 1.4	NS	Yang *et al* (2013)
IL18	-1297/ -607/ -380/ -137/ +105 haplotype	Cervical cancer (CSCC)	470	Northern Taiwan	16, 18, other		NS	Yang *et al* (2013)
TNF	-1042 C > A (rs1800630)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	-1036 T > C (rs1799724)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
TNF	-487 A > G (rs1800629)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
TNF	-417 A > G (rs361525)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
TNF	-308	Cervical cancer HPV$^+$	42	Brazilian		GG: 73.7 AG/AA: 26.4	NS	Fernandes *et al* (2008)
TNF	-863 C > A	Cervical cancer HPV16$^+$	141	Hispanic	16	CC: 73.7 AC: 24.6 AA: 1.8	Genotype distribution significantly different between HPV16$^+$ controls and cases (P= .007)	Deshpande *et al* (2005)
TNF	-863 C > A	Cervical cancer HPV16$^+$	200	Non- Hispanic whites	16	CC: 76.5 AC: 21.0 AA: 2.5	NS	Deshpande *et al* (2005)
TNF	-857 C > T	Cervical cancer HPV16$^+$	141	Hispanic	16	CC: 73.0 TC: 22.6 TT: 4.4	Genotype distribution significantly different between random controls and cases (P= .043)	Deshpande *et al* (2005)
TNF	-857 C > T	Cervical cancer HPV16$^+$	200	Non- Hispanic whites	16	CC: 77.5 TC: 19.2 TT: 3.3	NS	Deshpande *et al* (2005)
TNF	-575 G > A	Cervical cancer HPV16$^+$	141	Hispanic	16	GG: 100.0 AG: 0.0 AA: 0.0	NS	Deshpande *et al* (2005)
TNF	-575 G > A	Cervical cancer HPV16$^+$	200	Non- Hispanic whites	16	GG: 100.0 AG: 0.0 AA: 0.0	NS	Deshpande *et al* (2005)
TNF	-572 A > C	Cervical cancer HPV16$^+$	141	Hispanic	16	AA: 91.4 CA: 8.6 CC: 0.0	NS	Deshpande *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	-572 A > C	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	AA: 94.0 CA: 5.0 CC: 1.0	Genotype distribution significantly different between random controls and cases (P=.047)	Deshpande *et al* (2005)
TNF	-568 C > G	Cervical cancer HPV16^{+}	141	Hispanic	16	CC: 97.5 GC: 1.2 GG: 1.2	NS	Deshpande *et al* (2005)
TNF	-568 C > G	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	CC: 100.0 GC: 0.0 GG: 0.0	NS	Deshpande *et al* (2005)
TNF	-376 G > A	Cervical cancer	127	American		GG: 95.3 AG: 4.7 AA: 0.0	For the -376 polymorphism, the A (variant) allele was identified in 0% of the cases and 4.7% (5 of 108) of the controls, or 2.3% (5 of 216) of the total alleles analyzed. The higher prevalence of TNF -376 HETs in the control group was statistically significant (P = 0.02).	Calhoun *et al* (2002)
TNF	-375 G > A	Cervical cancer HPV16^{+}	141	Hispanic	16	GG: 95.6 AG: 4.4 AA: 0.0	NS	Deshpande *et al* (2005)
TNF	-375 G > A	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	GG: 99.2 AG: 0.8 AA: 0.0	NS	Deshpande *et al* (2005)
TNF	-375 G > A	CIN 3/invasive carcinoma	56	Posadas (Misiones, Argentina)		GG: 91.2 AG: 8.8 AA: 0	TNF-375A SNP was identified in 8.8% of the controls and none of the cases. Moreover, the TNFA-375A always occurred in association with the TNFA-237A SNP, indicating linkage disequilibrium between them.	Badano *et al* (2012)
TNF	-308 G > A	Cervical cancer	195	North Portuguese	N/A	GG: 82.0 AG: 16.4 AA: 1.6	Women carrying the A allele present a twofold increased risk of developing ICC (p = 0.006; OR = 1.88; 95% CI [1.20–2.94]).	Duarte *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	-308 G > A	Cervical (pre)cancer	165	Indo-Aryan	16, 18	GG: 91 AG + AA: 9	–308 A allele in TNF was significantly higher in cases compared with control subjects (21% vs. 9%; OR= 2.7; 95% CI = 1.41–5.15; p < 0.01). A allele leads to 3 times increased susceptibility to HPV 16 (24% in HPV positive cases vs. 9% in controls; p < 0.01; OR = 3.1; 95% CI = 1.60–6.03).	Kohaar *et al* (2007)
TNF	-308 G > A	CIN1 CIN2/3	19 58	British		GG: 52 AG + AA: 48	All categories of CIN are associated with TNF -308 low secretor phenotype GG compared to women with normal cervical cytology. The association is strongest for CIN1, and 95% of CIN1 patients (18/19) are GG low secretors (P = 0.004), but only just reaches significance for the CIN2/3 group (P = 0.05).	Kirkpatrick *et al* (2004)
TNF	-308 G > A	Cervical cancer	244	South African		GG: 76 AG: 20 AA: 4	NS	Govan *et al* (2006)
TNF	-308 G > A	Cervical cancer	103	Zimbabwean		GG: 80 AG: 18 AA: 2	NS	Stanczuk *et al* (2003)
TNF	-308 G > A (rs1800629)	Cervical tumours (severe dysplasia, carcinoma in situ, invasive cancer)	1306	Swedish		GG: 71.74 AG: 25.0 AA: 3.26	NS	Ivansson *et al* (2010)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	-308 G > A	Cervical Cancer	127	American		GG: 68.2 AG: 38.0 AA: 3.8	NS	Calhoun *et al* (2002)
TNF	-308 G > A	Cervical cancer	186	Chinese		GG: 72 AG + AA: 28	NS	Wang *et al* (2011)
TNF	-308 G > A	Oa Cervical cancer	51	Pusan, South Korea		GG: 92.4 AG: 7.6 AA: 0.0	NS	Jang *et al* (2001)
TNF	-308 G > A	Cervical cancer	122	Argentinian (La Plata)	hrHPV lrHPV	GG: 71.6 GA: 26.1 AA: 2.3	NS	Barbisan *et al* (2012)
TNF	-308 G > A	CIN Invasive carcinoma	78	Romanian		GG: 77.6 GA: 21.5 AA: 0.9	Association was found between the presence of an A allele and invasive carcinoma when compared against the HPV positive controls (p=0.04, OR=10.83, 95% CI=1.07-109.05). The frequency of the allele was however low, as was the number of cases of invasive carcinoma.	Rotar *et al* (2014)
TNF	-307 G > A	Cervical cancer HPV16^{+}	141	Hispanic	16	GG: 73.4 AG: 23.9 AA: 2.6	NS	Deshpande *et al* (2005)
TNF	-307 G > A	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	GG: 72.3 AG: 25.2 AA: 2.5	NS	Deshpande *et al* (2005)
TNF	-307 G > A	CIN 3/invasive carcinoma	56	Posadas (Misiones, Argentina)		GG: 89.4 AG: 10.6 AA: 0	TNFA-307A was associated with cervical cancer development with an OR of 2.4 (1.1–5.4).	Badano *et al* (2012)
TNF	-244 G > A	Cervical Cancer	127	American		GG: 99.1 AG: 0.9 AA: 0.0	NS	Calhoun *et al* (2002)[22]
TNF	-243 G > A	Cervical cancer	141	Hispanic	16	GG: 97.4 AG: 1.7 AA: 0.9	NS	Deshpande *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	-243 G > A	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	GG: 99.2 AG: 0.8 AA: 0.9	NS	Deshpande *et al* (2005)
TNF	-243 G > A	CIN 3/invasive carcinoma	56	Posadas (Misiones, Argentina)		GG: 100.0 AG: 0	NS	Badano *et al* (2012)
TNF	-238 G > A	Cervical (pre)cancer	165	Indo-Aryan	16,18	GG: 96 AG + AA: 4	NS	Kohaar *et al* (2007)
TNF	-238 G > A	CIN1 CIN2/3	19 58	British		GG: 89 AG + AA: 11	NS	Kirkpatrick *et al* (2004)
TNF	-238 G > A	Cervical Cancer	127	American		GG: 88.8 AG: 11.2 AA: 0.0	TNFA -238 polymorphism was significantly underrepresented in cervical cancer patients [heterozygotes (HETs), OR = 0.33; 95% CI = 0.11– 0.96]	Calhoun *et al* (2002)
TNF	-238 G > A	Oa Cervical cancer	51	Pusan, South Korea		GG: 86.9 AG: 12.0 AA: 1.1	NS	Jang *et al* (2001)
TNF	-238 G > A	Cervical cancer	122	Argentinian (La Plata)	hrHPV lrHPV	GG: 84.7 GA: 15.3 AA: 0.0	Heterozygous genotype accounts for a slightly lower risk of cervical cancer. The significance was borderline, P=0.069, OR=0.42.	Barbisan *et al* (2012)
TNF	-237 G > A	Cervical cancer HPV16^{+}	141	Hispanic	16	GG: 87.9 AG: 12.1 AA: 0.0	NS	Deshpande *et al* (2005)
TNF	-237 G > A	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	GG: 93.6 AG: 6.6 AA: 0.0	NS	Deshpande *et al* (2005)
TNF	-237 G > A	CIN 3/invasive carcinoma	56	Posadas (Misiones, Argentina)		GG: 96 AG: 14.2 AA: 0.9	NS	Badano *et al* (2012)
TNF	-161 C > T	Cervical cancer	141	Hispanic	16	CC: 98.2 TC: 1.8	NS	Deshpande *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
		HPV16^{+}				TT: 0.0		
TNF	-161 C > T	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	CC: 100.0 TC: 0.0 TT: 0.0	NS	Deshpande *et al* (2005)
TNF	-76 T > A	Cervical cancer HPV16^{+}	141	Hispanic	16	TT: 99.1 AT: 0.0 AA: 0.9	NS	Deshpande *et al* (2005)
TNF	-76 T > A	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16	TT: 99.1 AT: 0.0 AA: 0.9	NS	Deshpande *et al* (2005)
TNF	Haplotypes -161/-237/ -307/-375/ -572/-575 -857/-863	Cervical cancer HPV16^{+}	141	Hispanic	16		Haplotype TN*01(-161C/-237G/-307G/-375G/-572A/-575G/-857C/-863C) was present at a significantly lower frequency in randomly selected control subjects than in case subjects (P=.001), whereas TN*04, defined by SNP -307, (-161C/-237G/-307A/-375G/-572A/-575G/-857C/-863C) was present at a significantly lower frequency in HPV16-positive control subjects than in case subjects (P= .017)	Deshpande *et al* (2005)
TNF	Haplotypes -161/-237/ -307/-375/ -572/-575 -857/-863	Cervical cancer HPV16^{+}	200	Non- Hispanic whites	16		TN*06 (defined by SNP -572), (-161C/-237G/-307G/-375G/-572C/-575G/-857C/-863C) was present at a significantly higher frequency in randomly selected control subjects than in case subjects (P=.004).	Deshpande *et al* (2005)
TNF	Haplotypes -375/-307/ -243/-237	CIN 3/invasive carcinoma	56	Posadas (Misiones, Argentina)		H1 (G/G/G/G): 86.7 H2 (A/G/G/A): 4.4 H3 (G/A/G/G): 5.3 H4 (G/G/A/G): 0 H5 (G/G/G/A): 3.5	Haplotype A/G/G/A was present only in controls (p = 0.03). Haplotype G/A/G/G was present at a significantly higher frequency in case subjects compared to controls (p 0.03).	Badano *et al* (2012)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	TNFa microsatellite polymorphism a1:(AC/ GT)$_6$ a2:(AC/ GT)$_7$ . . . a13:(AC/GT)$_{18}$	CIN	64	Swedish	16,18	1$^{\#}$: 2 2: 46 3: 1 4: 16 5: 9 6: 34 7: 4 8: 1 9: 4 10: 15 11: 39 13: 3	The TNFa-11 allele was significantly more frequent in HPV16 seropositive patients compared with seronegative patients (OR, 5.40; Pc , 0.01; 95% CI, 1.9 –15.3), but this significant finding was not observed in HPV18-positive patients.	Ghaderi *et al* (2000)
TNF	TNFa microsatellite polymorphism	Cervical cancer	85	Swedish	16,18	2$^{\#}$: 33 4: 15 5: 19 6: 40 10: 18 11: 41	TNFa-11 frequency was increased in the HPV18 DNA positive patients (OR = 2.84, p =0.0481, CI = 1.04 –7.78, pc = NS). TNFa-11 was not associated with susceptibility to HPV16 infection	Ghaderi *et al* (2001)
TNF	TNFa microsatellite polymorphism	SIL	146	Brazilian	16,18	1$^{\#}$: 3 2: 20.3 3: 3.5 4: 6.4 5: 5.4 6: 19.3 7: 15.3 8: 0 9: 2.4 10: 12.4 11: 8 12: 1.5 13: 2.5 14: 0	Significant associations were observed between LSIL and the TNFa-8 allele (4/166; P = 0.04), as well as between TNFa-2 with HPV18 only (16/44; P 0.002) and TNFa-2 with HPV18 coinfection with HPV16 (16/44; P = 0.001). Patients exhibiting the TNFa-2 allele and harboring HPV18, in the presence or absence of coinfection with HPV16, had an increased risk of HSIL occurrence (13/38; P = 0.04; 5/10; P 0.04) compared to patients with other HPV types.	Simoes *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TNF	TNFb microsatellite polymorphism	SIL	146	Brazilian	16,18	1: 17.3 2: 0 3: 9 4: 33.7 5: 36.1 6: 0.5 7: 3.4	NS	Simoes *et al* (2005)
TNF	TNFc microsatellite polymorphism	SIL	146	Brazilian	16,18	1: 66.4 2: 33.6	NS	Simoes *et al* (2005)
TNF	TNFd microsatellite polymorphism	SIL	146	Brazilian	16,18	1: 0.5 2: 8.4 3: 5.4 4: 50 5: 23.2 6: 11 7: 1.5	NS	Simoes *et al* (2005)
TNF	TNFe microsatellite polymorphism	SIL	146	Brazilian	16,18	1: 15.8 2: 1.5 3: 82.7	NS	Simoes *et al* (2005)
TNF	Haplotype TNFa-11/ HLA-DQ6	CIN	64	Swedish	16,18		Maximum risk in HPV16 seropositive patients with the haplotype TNFa-11 and HLA-DQ6.	Ghaderi *et al* (2000)
TNF	Haplotype TNFa-11/ HLA-DQ6	Cervical cancer	85	Swedish	16,18		The HLA DQ6-TNFa-11 extended haplotype increases the risk for cervical cancer three times (OR = 3.08, CI = 1.30–7.31, p = 0.0104). Infection with HPV 16 in the patients with HLA DQ6-TNFa-11 haplotype (DQA 1*0102–DQB 1*0602) is increasing the risk for the cancer of cervix by 23 fold (OR = 23.03, CI = 1.30–409.16, p = 0.0018).	Ghaderi *et al* (2001)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
LTA	804 C > A (rs1041981)	Cervical cancer (SCC, ADSC, ACC)	131	Japanese		CC: 33.4 AC: 51.6 AA: 15.0	Compared with the 804CC genotype, 804CA and 804AA were associated with a decreased risk of cervical cancer (ORZ0.64, 95% CIZ0.40–1.02; and ORZ0.45, 95% CIZ0.21–0.95, respectively), especially of SCC (OR 0.54, 95% CI 0.32–0.91; and OR 0.39, 95% CI 0.16–0.92, respectively). Among ADC/ADSC cases, there was no significant association between the LTa C804A polymorphism and cancer risk.	Niwa *et al* (2005)
LTA	252 A > G (rs909253)	Cervical cancer	131	Japanese		AA: 33.4 GA: 51.6 AA: 15.0	*Complete linkage disequilibrium*	Niwa *et al* (2005)
LTA	+90 A > G (rs909253)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
LTA	+49 A > C Or +80 A > C (rs2239704)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IFNG	-1615 C > T (rs2069705)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IFNG	rs11177074	CIN3/cervical cancer Persistent HPV	416 356	Costa Rican			Associated with progression to precancer/cancer ($P = 0.0003$)	Wang *et al* (2010)
IFNG	+874 T > A (rs2430561)	SCC AC	175 25	North Indian	N/A	TT: 12.5 AT: 37.5 AA: 50.0	Genotypes AT and AA + AT increase the risk of cervical cancer (OR = 3.3, 95% CI — 2.05–5.2, P ≤ 0.001 — OR = 2.9, 95% CI — 1.9–4.6, P ≤ 0.001, respectively).	Kordi Tamandani *et al* (2008)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IFNG	+874 T > A	Cervical tumours (severe dysplasia, carcinoma in situ, invasive cancer)	1306	Swedish		TT: 34 AT: 46 AA: 20	NS	Ivansson *et al* (2010)
IFNG	+874 T > A	Cervical cancer + HPV^{+}	42	Brazilian		TT: 18.4 AT: 32.2 AA: 49.4	NS	Fernandes *et al* (2008)
IFNG	+874 T > A	Cervical Cancer	200	North Indian		TT: 19.6 AT: 50.0 AA: 30.4	IFN-c AA genotype which is low producer of IFN-c was associated with increased risk of cervical cancer (OR = 2.43, P = 0.003). Allele A was at 1.54-fold increased risk of cervical cancer (OR=1.54, P = 0.002). The AA genotype showed statistically significant risk with high stage (3 + IV) of cervical cancer (OR = 4.99, P = 0.001). In tobacco users, AA genotype showed significantly increased susceptibility to cervical cancer (OR = 5.08, P = 0.010).	Gangwar *et al* (2009)
IFNG	+874 T > A	Invasive squamous cell carcinoma	82 83 64	Brazilian Whites Whites Non-Whites			NS	Guzman *et al* (2008)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IFNG	+874 T > A	Cervical cancer	186	Chinese		TT: 19.0 AT: 48.0 AA: 33.0	The IFN-g+874A/A genotype was associated with high risk for the development of cervical cancer [odds ratio (OR) 2.22, p = 0.012], and the A allele was associated with a 1.47-fold increased risk of cervical cancer (p = 0.009).	Wang *et al* (2011)
IFNG	Genotype combination CTLA4-19 C > T IFNG+874 T > A	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		See single SNPs	Genotype combination CTLA4-319 (CC)/IFNG+874(AA) was associated with reduced cervical cancer susceptibility with OR=0.67 (0.53–0.84), p=0.0005, pemp=0.0007.	Ivansson *et al* (2010)
IFNG	Promotor CA repeats	LSIL HSIL SCC	93 123 153	Taiwanese		Rep[#] / Ctrl LSIL / Ctrl HSL / Ctrl SCC 11 / 1 / 0 / 0 12 / 17 / 12 / 16 13 / 39 / 44 / 42 14 / 1 / 1 / 0 15 / 35 / 35 / 34 16 / 2 / 2 / 2 17 / 1 / 0 / 1 18 / 5 / 5 / 5	Genotypes containing the 12 or 14 alleles were more common in HSILs than in controls (46% vs. 22%; OR 3.0; 95% CI 1.7–5.2; p < 0.0001). 13 and 18 were more common in controls than in HSILs (76% vs. 53%; OR 0.3; 95% CI 0.2– 0.6; p 0.0001) or SCC (74% vs. 63%; OR 0.6; 95% CI 0.4 –1.0; p 0.037). The frequency of the 12 and 14 genotypes increased with the severity of cervical carcinogenesis (ptest for trend 0.0002), whereas the 13 and 18 genotypes showed the opposite trend (ptest for trend 0.007).	Lai *et al* (2005)
IFNA17	Ile184Arg (exon 35 T > G)	Cervical squamous cell carcinoma	173	Korean		Arg/Arg: 32 Arg/Ile: 56 Ile/Ile: 12	Cancer patients were more likely than the control subjects to carry the 184Ile allele (0.509 vs. 0.399, P = 0.0041).	Kim *et al* (2003)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
Anti-inflammatory cytokines								
IL1RN	+59 A > T (rs454078)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL1RN	86 bp VNTR Alleles: A: 4 repeats B: 2 repeats C: 5 repeats D: 3 repeats	SCC, AC	150	North Indian	16, 18	AA: 45.0 AB: 43.5 BB: 6.2 AC: 2.4 BC: 1.9 DD: 0.5 DA: 0.5 DB: -	There was a strong significantly protective association between heterozygous *AB* genotype and HPV 18 (OR = 0.11, 95% CI = 0.04–0.30, *p* = 0.0000000). Similarly this result was demonstrated, in combined *AB* + *BB* genotypes of *IL-1RA* with HPV 18 (OR = 0.12, 95% CI = 0.05–0.30, *p* = 0.0000000) and HPV type 16 + 18 (OR = 0.18,95% CI = 0.08–0.38, *p* = 0.000005).	Kordi Tamandani *et al* (2007)
IL1RN	86 bp VNTR Alleles: 1: 4 repeats 2: 2 repeats 3: 5 repeats 4: 3 repeats	Cervical Cancer	404	Chinese Han		1/1: 87.1 1/2: 11.6 1/3: 0.2 1/4: 0.5 2/2: 0.5	NS	Qian *et al* (2010)
IL1RN	86 bp VNTR Alleles: 1: 4 repeats 2: 2 repeats 3: 5 repeats 4: 3 repeats 5: 6 repeats	Cervical cancer	150	North Indian		1/1: 63.58 1/2: 23.45 2/2: 6.17 1/3: 0.61 3/3: 2.46 1/4: 2.46 1/5: 0.60 5/5: 0.61	IL-1RN genotypes 1/2 and 2/2 were associated with significantly elevated risk of cervical cancer (OR = 3.3; P = 4.9 · 10–6 and OR = 2.9, P = 0.02). 2/2 genotype of IL-1RN was associated with higher stages of cervical cancer (OR = 4.8, P = 0.0006).	Singh *et al* (2008)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL1RN	VNTR intron2	CIN 2/3	131	Austrian Caucasian		Long/long: 45.8 Long/2: 38.2 2/2: 16.0	NS	Grimm *et al* (2011)
IL1RN	Haplotype 86 bp VNTR/IL1B -511 C > T	Cervical cancer	150	North Indian		1C: 31.48 1T: 45.98 2C: 6.48 2T: 11.41 3T: 2.77 4C: 0.30 4T: 0.92 5T: 0.92	1T (IL-1RN*1/IL-1b*T) and 2T (IL-1RN*2/IL-1b*T) were associated with the risk of cancer (OR = 1.7; P = 0.011 and OR = 4.08; P = 1.0 · 10–6). Both haplotypes were also associated with increased risk of cervical cancer for stage 3.	Singh *et al* (2008)
IL10	-3584 A > T (rs1800890)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL10	-1352 G > A (rs1800893),	Squamous intraepithelial cervical lesions	204	Mexican		GG: 61.44 AG: 31.94 AA: 6.62	NS	Torres-Poveda *et al* (2012)
IL10	-1116 A > G (rs1800896)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL10	-1082 G > A (rs1800896)	$hrHPV^+$ +normal smear $hrHPV^+$ + CIN1 $hrHPV^+$ + CIN2/3	54 15 47	British	hrHPV	GG: 21 AG: 52 AA: 27	Significant increase in HPV clearance among women with normal cytology who carried at least one G allele.	Farzaneh *et al* (2006)
IL10	-1082 G > A	Cervical cancer	77	Zimbabwe, Shona		GG: 0 AG: 16 AA: 84	Cases were more likely to be predisposed to produce higher (A/G) levels of IL-10 (p = 0.001). The genotype encoding for high (G/G) production of IL-10 was only observed in one cancer	Stanczuk *et al* (2001)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
							patient. Low producers of IL-10 were less frequent in cases than in controls. There were no high producers amongst the healthy women. These data suggest that the genetically acquired ability to produce higher levels of IL-10 may be a significant factor in the development of cervical cancer.	
IL10	-1082 G > A	CIN Invasive cervical cancer	163 104	Japanese		GG: 0.6 AG: 9.2 AA: 90.2	The carrier frequency of interleukin-10 -1082 G alleles associated with higher interleukin-10 production increased with disease severity: 9.8% for normal cytology; 19.6% for cervical intraepithelial neoplasia; 29.8% for invasive cervical cancer (P for trend = 0.001)	Matsumoto *et al* (2010)
IL10	-1082 G > A	Cytologic or colposcopic abnormalities, P3	253	Hungarian		AA: 25 GA: 51 GG: 24	NS	Szöke *et al* (2004)
IL10	-1082 G > A	CIN Cervical cancer	311 695	Dutch		AA: 27.9 GA: 50.7 GG: 21.5	NS	Zoodsma *et al* (2005)
IL10	-1082 G > A	Invasive cervical cancer	144	Korean		AA: 100 GA: 0 GG: 0	NS	Roh *et al* (2002)
IL10	-1082 G > A	Cervical cancer	186	Chinese		GG: 10.5 AG: 38.0 AA: 51.5	NS	Wang *et al* (2011)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL10	-1082 G > A (rs1800896)	Squamous intraepithelial cervical lesions	204	Mexican		AA: 55.4 GA: 37.3 GG: 7.3	NS	Torres-Poveda *et al* (2012)
IL10	-1082 G > A	Cervical cancer	122	Argentinian (La Plata)	hrHPV lrHPV	AA : 44.9 GA : 47.2 GG : 8.0	NS	Barbisan *et al* (2012)
IL10	-1082 A/G (rs1800896)	Cervical lesions, HPV infection	171	Brazilian	16, other	AA : 36 AG : 47 GG : 17	There was a significant association between G allele/GG genotype and an increase in risk for susceptibility, development and progression of cervical lesions in HPV+ women that use oral contraceptives (OR = 1.71, CI 1.10–2.76, p = 0.0162; and OR = 2.51, CI 1.02–6.15, p = 0.0332, respectively).	Chagas *et al* (2013)
IL10	-853 C > T (rs1800871)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL10	-819 C > T (rs1800871)	Squamous intraepithelial cervical lesions	204	Mexican		CC: 34.3 TC: 45.2 TT: 20.5	NS	Torres-Poveda *et al* (2012)
IL10	-819 C > T (rs1800871)	Invasive cervical cancer	144	Korean		CC: 8 TC: 43 TT: 49	NS	Roh *et al* (2002)
IL10	-819 C/T (rs1800871)	Cervical lesions, HPV infection	171	Brazilian	16, other	CC : 39 CT : 45 TT : 16	NS	Chagas *et al* (2013)
IL10	-626 A > C (rs1800872)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL10	-592 C > A (rs1800872)	Squamous intraepithelial cervical lesions	204	Mexican		CC: 40 AC: 42 AA: 18	Individuals carrying at least one copy of risk allele A of polymorphism −592 had a two-fold increased risk of developing SICL. The IL-10 mRNA expression and serum IL-10 protein, were significantly higher in SICL cases ($p < 0.01$), being higher in patients carrying the risk allele A.	Torres-Poveda *et al* (2012)
IL10	-592 C > A (-571) (rs1800872)	Severe dysplasia, in situ or invasive cervical cancer	1,306	Swedish			NS	Ivansson *et al* (2007)
IL10	-592 C > A (rs1800872)	CIN Cervical cancer	311 695	Dutch		CC: 66.8 CA: 28.9 AA: 4.3	Increased CIN (2–3) (OR 1.44 [1.06–1.97]) and squamous cell carcinoma of the cervix (OR 1.35 [1.04–1.75]) for individuals heterozygous for the A-allele of the IL-10 -592 polymorphism.	Zoodsma *et al* (2005)
IL10	-592 C > A	Invasive cervical cancer	144	Korean		CC: 8 CA: 43 AA: 49	NS	Roh *et al* (2002)
IL10	+210 T > C (rs3024496)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL10	Haplotype −1082/ −819 / −592	CIN Cervical cancer	311 695	Dutch		GCC: 52.9 ACC: 27.9 ATA: 18.9	NS	Zoodsma *et al* (2005)
IL10	Haplotype −1082/ −819 / −592	Invasive cervical cancer	144	Korean		Only 2 variants observed: ACC ATA	NS	Roh *et al* (2002)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls		Result	Reference
IL10	Haplotype −1082/ −819 / −592	Cervical cancer + HPV^{+}	42	Brazilian		AA/CC/CC, AA/CT/CA, AA/TT/AA: low producer	47.1	NS	Fernandes *et al* (2008)
						GA/CC/AC, GA/CT/CA: intermediate producer	43.7		
						GG/CC/CC: high producer	9.2		
TGFB	Haplotype Codon 10 & 25	Cervical cancer + HPV^{+}	42	Brazilian		CC/GC, CC/CC, TT/CC, TC/CC: low producer	5.7	NS	Fernandes *et al* (2008)
						CC/GG, TT/GC: intermediate producer	18.4		
						TT/GG, TC/GG: High producer	75.9		
TGFB	Haplotype Codon 10 & 25	Cervical cancer	186	Chinese		CC/GC, CC/CC, TT/CC, TC/CC: low producer	6.5	NS	Wang *et al* (2011)
						CC/GG, TT/GC: intermediate producer	18.5		
						TT/GG, TC/GG TC/GC: high producer	75		
TGFB1	Codon 10	SCC	93	Zimbabwean		TT: 56 CT: 23 CC: 12		NS	Stanczuk *et al* (2002)
TGFB1	Codon 25	SCC	93	Zimbabwean		GG: 84 CG: 16 CC: 0		NS	Stanczuk *et al* (2002)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TGFB1	-327 C > T (rs1982073)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
Chemokines								
CXCL12 (SDF-1)	rs17885289	Cervical Cancer	917	western Washington State		GG: 50.1 AG: 41.2 AA: 8.7	NS	Maley *et al* (2009f)
CXCL12 (SDF-1)	rs2839685	Cervical Cancer	917	western Washington State		CC: 72.9 TC: 25.7 TT: 1.3	NS	Maley *et al* (2009)
CXCL12 (SDF-1)	rs17880313	Cervical Cancer	917	western Washington State		CC: 76.6 TC: 21.7 TT: 1.7	NS	Maley *et al* (2009)
CXCL12 (SDF-1)	rs7092453	Cervical Cancer	917	western Washington State		AA: 65.6 GA: 30.6 GG: 3.7	NS	Maley *et al* (2009)
CXCL12 (SDF-1)	rs2236534	Cervical Cancer	917	western Washington State		CC: 65.9 AC: 29.5 AA: 4.5	NS	Maley *et al* (2009)
CXCL12 (SDF-1)	rs266085	Cervical Cancer	917	western Washington State		GG: 40.6 AG: 44.4 AA: 15.0	The minor (A) allele was inversely associated with cervical cancer under a recessive genetic effects model (OR=0.74, 95% C.I. 0.56–0.98).	Maley *et al* (2009)
CXCL12 (SDF-1)	rs1801157	Cervical Cancer	917	western Washington State		GG: 63.8 AG: 33.4 AA: 2.8	NS	Maley *et al* (2009)
CXCL12 (SDF-1)	rs3740085	Cervical Cancer	917	western Washington State		GG: 56.5 CG: 36.7 CC: 6.8	NS	Maley *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CXCL12 (SDF-1)	rs266093	Cervical Cancer	917	western Washington State		CC: 40.2 GC: 47.1 GG: 12.7	The minor (G) allele was associated with a weakly increased risk of cervical cancer (dominant model OR=1.11, 95% C.I. 0.92–1.35, recessive model OR=1.23, 95% C.I. 0.94–1.63, and log additive model OR=1.12, 95% C.I. 0.97–1.28)	Maley *et al* (2009)
CXCL12 (SDF-1)	Haplotype rs17885289 G > A / rs266085 G > A / rs266093 C > G	Cervical Cancer	917	western Washington State		GAC: 27.0 GGC: 26.1 AGG: 18.8 GGG: 17.5 AAC: 10.4	Subset of SNPs (5' flanking SNP rs17885289, intronic SNP rs266085, and 3' UTR SNP rs266093), the inferred minimal haplotype associated with risk was defined by minor alleles at rs17885289 and rs266085, and the common allele at rs266093	Maley *et al* (2009)

Table 3. *Overview of polymorphisms in genes coding for receptors*

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls			Result	Reference
Receptors										
CCR2	V64I G > A (rs1799864)	Severe dysplasia, *in situ* or invasive cervical cancer	1,306	Swedish					The CCR-2 64I variant was associated with decreased risk of cervical cancer; homozygote carriers of the 64I variant had an odds ratio of 0.31 (0.12–0.77). This association was detected in both carriers and non-carriers of the HLA DQB1*0602 cervical cancer risk allele.	Ivansson *et al* (2007)
CCR2	V64I G > A	Cervical Cancer	446	Black African (106), mixed ancestry (340)		GG AG AA	Black 62 37 1	Mixed-ancestry 66 33 1	The CCR2-64I variant was significantly associated with cervical cancer when cases were compared to the control group (P = 0.001). The polymorphism does not affect the susceptibility to HPV infection or HSIL in South African women of black and mixed-ancestry origin. This result implies that the role of CCR2 is important in invasive cancer of the cervix but not in HPV infection or in the development of pre-cancers.	Chatterjee *et al* (2010)
CCR2	-64	SIL ICC	109 217	Portuguese		GG AG AA	SIL 78.0 22.0 0.0	ICC 88.9 10.6 0.5	G/A genotype was significantly higher in SIL patients than ICC patients (P = 0.005; OR = 0.42; 95% CI: 0.22–0.83). Significant association was found in the progression from high-grade SIL to ICC (OR = 0.435; 95% CI = 0.222–0.854; P = 0.014).	Coelho *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CCR2	CCR2-64I	CIN Cervical cancer	50 100	Swedish	N/A	GG: 75.7 AG: 23.0 AA: 1.3	NS	Zheng *et al* (2006)
CCR5	CCR5-Δ32	CIN Cervical cancer	50 100	Swedish	N/A	WT/WT: 88.6 Δ32/WT: 10.7 Δ32/ Δ32: 0.67	A slight HPV infection risk for CCR5-¢32 homozygous mutant carriers was significant (OR=4.58; p=0.045)	Zheng *et al* (2006)
CD28	+17 T > C	Invasive squamous cell carcinoma	82 83 64	Brazilian Whites Non-Whites			NS	Guzman *et al* (2008)
CD28	+17 T > C	CSCC	147	Polish caucasian			NS	Pawlak *et al* (2009)
CD28	+17 T > C (rs3116496)	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		TT: 65.83 CT: 31.47 CC: 2.70	NS	Ivansson *et al* (2010)
CD28	Haplotype CD28 +17 T > C + IFNG +874 T > A	Invasive squamous cell carcinoma	82 83 64	Brazilian Whites Whites Non-Whites			Overall frequency of CD28(TT)/ IFNG(AA) in patients and controls (229 patients and 193 controls) showed higher frequency of this genotype combination in patients (35%) than in controls (20%) [Fisher's exact test: P ¼ 0.0011; (OR) ¼ 2.07, 95% CI: 1.32–3.24].	Guzman *et al* (2008)
CD28	Genotype combination CD28+17 T > C IFNG+874 T > A	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		See single SNPs	The genotype combination CD28+17 (TT) and IFNG+874 (AA) was less common in cases than controls with an Odds Ratio=0.76 (95% CI 0.60–0.96), p=0.02 (uncorrected), pemp=0.03	Ivansson *et al* (2010)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CD28	Genotype combination CD28+17 T > C IFNG+874 T > A TNF-308 G > A	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		See single SNPs	NS	Ivansson *et al* (2010)
CD28	Genotype combination CD28+17 T > C IFNG+874 T > A PDCD1+7785 C > T	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		See single SNPs	NS	Ivansson *et al* (2010)
CD28	Genotype combination CD28+17 T > C IFNG+874 T > A ICOS+1564 T > C	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		See single SNPs	Combination of CD28+ 17(TT)/IFNG+874(AA) /ICOS+1564(TT) was associated with decreased risk with OR=0.65 (0.49–0.87), p=0.004, pemp=0.006).	Ivansson *et al* (2010)
CD28	Haplotypes CD28+17 T > C CTLA4-19 C > T ICOS+1564 T > C	Severe dysplasia, carcinoma in situ, invasive cancer	1306	Swedish		See single SNPs	NS	Ivansson *et al* (2010)
CD80	+35 G > A (rs2228017)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
CD83	rs12205252	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs6929821	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CD83	rs3799925	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs3799924	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs4715877	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs7743206	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs1050648	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs3734665	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs10949227	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs17354216	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs9296925	Cervical cancer	263	Eastern United States		TT: 31.3 CT: 51.9 CC: 16.7	NS	Yu *et al* (2009)
CD83	rs9296925	Cervical cancer CIN 3	255 122	American (Caucasian - 341, African-American – 36)	16,18		Associated with risk of HPV16+ or HPV18+ ICC (P = 0.0193) but not susceptibility to hrHPV infection of CIN 3	Zhang *et al* (2007)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CD83	rs9296925	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs853360	Cervical cancer	263	Eastern United States		CC: 51.1 TC: 42.6 TT: 6.3	NS	Yu *et al* (2009)
CD83	rs853360	Cervical cancer CIN 3	255 122	American (Caucasian - 341, African-American – 36)	16,18		Associated with risk of HPV16+ or HPV18+ ICC (P = 0.0035) but not susceptibility to hrHPV infection of CIN 3	Zhang *et al* (2007)
CD83	rs853360	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs853369	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD83	rs9230	Cervical cancer	263	Eastern United States		CC: 56.7 TC: 42.6 TT: 6.7	NS	Yu *et al* (2009)
CD83	rs9230	Cervical cancer CIN 3	255 122	American(Caucasian - 341, African-American – 36)	16,18		Associated with risk of HPV16+ or HPV18+ ICC (P = 0.0011) but not susceptibility to hrHPV infection of CIN 3	Zhang *et al* (2007)
CD83	rs9370729	Cervical cancer	263	Eastern United States		CC: 24.5 TC: 51.1 TT: 24.5	NS	Yu *et al* (2009)
CD83	rs9370729	Cervical cancer CIN 3	255 122	American (Caucasian - 341, African-American – 36)	16,18		Associated with risk of HPV16+ or HPV18+ ICC (P = 0.0012) but not susceptibility to hrHPV infection of CIN 3	Zhang *et al* (2007)
CD83	rs9370729	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CD83	rs750749	Cervical cancer	263	Eastern United States		TT: 54.9 CT: 38.7 CC: 6.4	Carriers of the CT or CC genotypes demonstrated a 30% and 50% reduction in disease risk, relative to carriers of the more common TT genotype (p-trend=0.02).	Yu *et al* (2009)
CD83	rs750749	Cervical cancer CIN 3	255 122	Washington (Caucasian - 341, African-American – 36)	16,18		Associated with risk of HPV16+ or HPV18+ ICC (P = 0.0133) but not susceptibility to hrHPV infection of CIN 3	Zhang *et al* (2007)
CD83	rs750749	SCC Adeno-carcinoma	390 469	Western Washington state			NS	Bodelon *et al* (2012)
CD86	-151 G > A (rs2681417)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
CD86	+35 G > A (rs1129055)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
CD279 (PDCD1)	+7785 C > T (rs2227981)	Cervical tumours (severe dysplasia, carcinoma in situ, invasive cancer)	1306	Swedish		CC: 31.7 TC: 46.40 TT: 21.9	NS	Ivansson *et al* (2010)
CTLA4	-1722 T > C	Cervical cancer	55	Iranian		TT: 81.8 TC: 18.2 CC: 0.0	NS	Rahimifar *et al* (2010)
CTLA4	- 1661 A > G	Cervical cancer	55	Iranian		AA: 67.3 AG: 28.2 GG: 4.5	The frequency of A/A homozygote and A allele were lower in patients than in controls. (P=0.01, P=0.035; respectively)	Rahimifar *et al* (2010)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CTLA4	(rs5742909)	CSCC	147	Polish Caucasian		CC: 83.3 TC: 16.2 TT: 0.5	The CTLA-4g.319CT[T] allele and individuals carrying T allele (TTCT genotype) were more frequent in CSCC patients compared with controls (p 0.003, OR 1.99, 95% CI 1.25–3.17, and p 0.005, OR 2.07, 95% CI 1.24–3.45, respectively)	Pawlak *et al* (2009)
CTLA4	-319 C > T (rs5742909)	Cervical tumours (severe dysplasia, carcinoma in situ, invasive cancer)	1306	Swedish		CC: 82.7 TC: 16.6 TT: 0.7	NS	Ivansson *et al* (2010)
CTLA4	-318 C > T (rs5742909)	HPV infection, cervical cancer	100	Indian	16, 18, 11	CC: 93.07 CT: 6.93 TT: 0.00	NS	Gokhale *et al* (2013)
CTLA4	-318 C > T	Cervical cancer	55	Iranian		CC: 80.9 TC: 18.2 TT: 0.9	At position −318 the frequency of C/C homozygote and C allele were increased in patients (P=0.021, P=0.025; respectively).	Rahimifar *et al* (2010)
CTLA4	-318 C > T	All SCC HPV 16+ SCC	144 80	Taiwanese	16, 18, other	CC: 81.0 TC: 17.7 TT: 1.3	C/T genotype is more frequent in women with HPV 16+ SCC (OR 1.99, 95%CI 1.16-3.42, *P* = 0.03) as compared to controls	Su *et al* (2007)
CTLA4	CTLA-4g .*642AT(8_33)	CSCC	147	Polish Caucasian		(AT)8/(AT)8: 26.7 (AT)8/(AT)>8: 40.4 (AT)>8/(AT)>8: 32.9	The CTLA-4g.*642AT(8_33) [(AT)8] allele was overrepresented in CSCC patients in comparison with healthy women (p 0.03, OR 1.41, 95% CI 1.04–1.90)	Pawlak *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CTLA4	-61A > G (rs231775)	CIN3/Cancer Persistent HPV	470 390	Costa Rican				Wang *et al* (2009)
CTLA4	+49 A > G	Cervical cancer	55	Iranian		AA: 52.7 GA: 40.9 GG: 6.4	NS	Rahimifar *et al* (2010)
CTLA4	+49 A > G	SCC	144	Taiwanese	16, 18, other		NS	Su *et al* (2007)
CTLA4	+49 A > G (rs231775)	CSCC	147	Polish caucasian			NS	Pawlak *et al* (2009)
CTLA4	+49 A > G (rs231775)	HPV infection, cervical cancer	104	Indian	16, 18, 11	AA; 17.66 AG: 53.70 GG: 29.63	Both the AA genotype and the A allele were significantly more present in cases (invasive cervical cancer with HPV infection) than in women with normal cytology and with or without HPV infection (P=0.01, OR=2.2, 95% CI= 1.2-4.2; and P=0.04, OR=1.5, 95% CI=1.0-2.1, respectively). The allelic frequency of G was conversely higher in controls than cases (P=0.04).	Gokhale *et al* (2013)
CTLA4	+6230 A > G	All SCC	144	Taiwanese	16, 18 other		NS	Su *et al* (2007)
CTLA4	+6230 A > G	CSCC	147	Polish caucasian			NS	Pawlak *et al* (2009)
CTLA4	Haplotypes -1722/-1661/-318/+49	Cervical cancer	55	Iranian		TGTA: 9.54 TACG: 18.18 TGCA: 9.09 TACA: 53.63 CACG: 8.63 TATA: 0.45 TGTG: 0.00 TGCG: 0.00	TGTA haplotype was only observed in control group (9.54%, p=0.002) and the TGTG and TGCG haplotypes only occurred in cervical cancer patients (2.77%, p=0.035; 6.48%, p=0.0003).	Rahimifar *et al* (2010)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
CTLA4	Haplotypes - 318/+49/+6230	SCC	144	Taiwanese	16, 18, other		NS	Su *et al* (2007)
CTL4	Haplotypes -318/+49	HPV infection, cervical cancer	N/A	Indian	16, 18, 11	AC: 0.42 AT: 0.02 GC: 0.55 GT: 0.003	NS	Gokhale *et al* (2013)
FAS	-1377 G > A	Cervical cancer	314	Chinese		GG: 45.8 AG: 45.1 AA: 9.1	NS	Sun *et al* (2005)
FAS	-1377 G > A	HSIL SCC	143 175	Chinese Han		GG: 31.1 AG: 51.9 AA: 17.0	NS	Lai *et al* (2005)
FAS	-1377 G > A	SCC	38	Spanish	16	GG: 26 AG: 72 AA: 2	NS	Lerma *et al* (2008)
FAS	-670 G > A	SCC	38	Spanish	16	GG: 26 AG: 51 AA: 23	A trend between GG genotype and adverse prognosis was observed (p= 0.065).	Lerma *et al* (2008)
FAS	-670 G > A	CIN Cervical cancer	311 695	Dutch		GG: 26.7 AG: 47.9 AA: 25.5	NS	Zoodsma *et al* (2005)
FAS	-670 G > A (rs1800682)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
FAS	-670 A > G	LSIL, HSIL*	167 49	Japanese	Hr-HPV	HPV- AA: 28.3 GA + GG: 71.7 HPV+ AA: 40.0 GA + GG: 60.0	49 patients with HSIL had higher frequency of hrHPV and GA + GG genotype than 167 with LSIL and 63 controls. There was an increased OR (6.00; 95% CI 1.32 – 27.37; p=0.021) for GA + GG genotype in HSIL cases compared to controls.	Ueda *et al* (2005)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
FAS	-670 A > G	Cervical cancer	314	Chinese		AA: 43.6 AG: 44.2 GG 12.2	NS	Sun *et al* (2005)
FAS	-670 A > G	HSIL SCC	143 175	Chinese Han		GG: 20.8 GA: 50.6 AA: 28.6	The A-allele and AA-genotype frequencies of FAS-670 A > G were significantly higher in HSIL/SCC than in controls (60% vs. 54%, P = 0.04, OR 1.26 [95% CI 1.01–1.57]; 38.0% vs. 28.6%, P = 0.02, OR 1.70 [95% CI 1.07–2.70]).	Lai *et al* (2005)
FAS	-670 A > G	LSIL HSIL SCC	104 131 176	Taiwanese		AA: 25.1 AG: 54.4 GG: 20.5	The frequency of the A allele was significantly (p = 0.006) higher in SCC than in control, conferring an odd ratio of 1.5 (95% CI = 1.1–2.0). The distribution of Fas (-670) genotypes also differed significantly between HSIL, SCC and each of their control (p = 0.017 and 0.03, respectively), with the A/A genotype conferring an OR of 1.3 (95% CI = 1.1–1.6) and 1.6 (95% CI = 1.0 –2.5), respectively. Remarkably, the frequency of A allele and A/A genotype increased gradually in accordance with the multi-step carcinogenesis from LSIL, HSIL to SCC (ptest for trend = 0.0066 and 0.0007, respectively).	Lai *et al* (2003)
FAS	Haplotype -1377 /-670	HSIL SCC	143 175	Chinese Han		N/A	The FAS -1377A/-670A haplotype conferred a higher risk for HSIL/SCC (OR 3.05, 95% CI 1.28–7.30) than FAS 670A alone (OR 1.26, 95% CI 1.28–7.30).	Lai *et al* (2005)
FAS	-252 C > T (rs1468063)	CIN3/Cancer	470 390	Costa Rican			NS	Wang *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
FAS	+16 G > A (rs3218619)	CIN3/Cancer	470 390	Costa Rican			NS	Wang *et al* (2009)
FASL	-844 T > C	Cervical cancer	314	Chinese		TT: 6.5 CT: 47.3 CC: 46.2	The FASL –844CC genotype had a threefold increased risk of developing cervical cancer OR (3.05; 95% CI 1.43–6.52; P= 0.004) compared with those carrying the TT genotype. The heterozygous CT genotype also presented a higher risk for the cancer OR(1.68; 95% CI 0.78–3.66), although the association was not statistically significant (P=0.187).	Sun *et al* (2005)
FASL	-844 T > C	HSIL SCC	143 175	Chinese Han		TT: 8.5 CT: 41.8 CC: 49.7	NS	Lai *et al* (2005)
FASL	-844 C > T (rs763110)	Severe dysplasia, in situ or invasive cervical cancer	1,306	Swedish			NS	Ivansson *et al* (2007)
FASL	Haplotype FAS -670 + FASL -844	HSIL SCC	143 175	Chinese Han			The interaction between FAS 670AA and FASL -844CC genotypes was associated with a risk of HSIL/SCC (OR 2.13, 95% CI 1.06–4.29) higher than that of the FAS -670AA genotype alone (OR 1.70, 95% CI 1.07–2.70).	Lai *et al* (2005)
FCGR2A (CD32)	-120 A > G (rs1801274)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
ICOS	+1564 T > C	CSCC	147	Polish Caucasian			NS	Pawlak *et al* (2009)
ICOS	+1564 T > C (rs4404254)	Cervical tumours (severe dysplasia, carcinoma in situ, invasive cancer)	1306	Swedish		TT: 60.98 CT: 33.76 CC: 5.26	NS	Ivansson *et al* (2010)
IFNGR2	-141 G > A (rs12655)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
	-134 C > T (rs1059293)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
	- 34 C > G (rs4986958)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
	- 16 A > G (rs9808753)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL4RA	I75V A > G (rs1805010)	Severe dysplasia, in situ or invasive cervical cancer	1,306	Swedish			IL-4R I75V showed a significant difference in the genotype distribution ($p < 0.01$) and allele frequencies ($p < 0.005$) between cases and controls. The G-allele, which corresponds to IL-4R 75V, was more common among cases than controls (46.2% compared to 39.3%) and thus associated with increased risk of cervical cancer.	Ivansson *et al* (2007)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL4RA	S503P T > C (rs1805015)	Severe dysplasia, in situ or invasive cervical cancer	1,306	Swedish			NS	Ivansson *et al* (2007)
IL4RA	Q576R A > G (rs1801275)	Severe dysplasia, in situ or invasive cervical cancer	1,306	Swedish			NS	Ivansson *et al* (2007)
IL4RA	Codon 576	Cervical cancer	127	American		A/A (Q/Q): 55.5 G/A (Q/R): 38.0 G/G (R/R): 6.5	NS	Calhoun *et al* (2002)
IL4RA	Haplotype I75V/ S503P/ Q576R	Severe dysplasia, in situ or invasive cervical cancer	1,306	Swedish		ATA: 48.0 GTA: 26.5 GCG: 11.7 ACG: 10.3 ATG: 2.3 GTG:1.2	The haplotype GTA was more common in cases than controls (p = 0.001).	Ivansson *et al* (2007)
IL8RA	+860 G > C (rs2234671)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL8RB	-1010 G > A (rs1126580)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL8RB	+811 C > T (rs2230054)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IL8RB	+1235 T > C (rs1126579)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
IL10RA	-109 G > A (rs9610)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
MBL1	Allele *0*: rs5030737 rs1800450 rs1800451 Allele *A*: wildtypes	SCC	172	Italian		A/A: 68 A/0: 26 0/0: 6	In HPV-infected women without cancer development, the presence of the O allele significantly associates with an increased risk of high-risk HPV infection (OR = 2.37, 95% CI = 1.45–3.89).	Segat *et al* (2009)
MBL2	rs11003125 H/L variant	hrHPV+	180	Brazilian		HH: 52.2 HL: 38.3 LL: 9.5	NS	Guimaraes *et al* (2008)
MBL2	rs7096206 X/Y variant	hrHPV+	180	Brazilian		XX: 17.2 XY: 40.6 YY: 42.2	NS	Guimaraes *et al* (2008)
MBL2	Haplotype rs5030737 rs1800450 rs1800451 allele A = WT/WT/WT allele 0 = MT/MT/MT	hrHPV+	180	Brazilian		AA: 65 A0: 32.8 00: 2.2	NS	Guimaraes *et al* (2008)
MBL2	Haplotype rs11003125 H/L rs7096206 X/Y (rs5030737 + rs1800450 + rs1800451) A/0	hrHPV+	180	Brazilian		High producer: 48.3 (HYA/HYA, HYA/LYA, HYA/LXA, LYA/LXA) Low producer: 42.2 (LXA/LXA, HYA/0, LYA/0) Deficient producer: 9.5 (LXA/0, 0/0)	When considering combined genotypes grouped according to MBL production, a significant difference was detected between healthy controls and high-risk HPV-positive patients, the latter group showing increased frequencies of deficient-producer genotypes (14.4% vs 9.4% in the healthy control group, corrected p	Guimaraes *et al* (2008)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
							0.04).	
NKG2C	WT/*null*(Δ)	LSIL, HSIL	572	Caucasian		WT/WT: 61.4 WT/*null*: 32.6 *null*/*null*: 6.0	NS (*null* deletion mutation does not represent a risk factor for HPV-induced cervical carcinogenesis)	Vilchez *et al* (2013)
TLR2	+613 T > C (rs3804099 rs3804100)	CIN3/Cancer Persistent HPV	470 390	Costa Rican		TT: 86 CT: 13 CC: 1	Decreased risk for CIN3 or cervical cancer (Ptrend value of .02) Associated with progression to CIN3 or cervical cancer.	Wang *et al* (2009)
TLR2	-196 to -174 del	Cervical cancer	150	North Indian		Ins/Ins: 76 Del/Ins: 23.3 Del/Del: 0.7	NS	Pandey *et al* (2009)
TLR4	+636 A > G (rs4986790)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
TLR4	+636 A > G (rs4986790)	Cervical cancer	150	North Indian		Asp/Asp: 82 Gly/Asp: 17.3 Gly/Gly: 0.7	NS	Pandey *et al* (2009)
TLR4	+936 C > T (rs4986791)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
TLR4	+936 C > T (rs4986791)	Cervical cancer	150	North Indian		Thr/Thr: 88.7 Ile/Thr: 10.6 Ile/Ile: 0.7	Ile/Thr genotype was associated with stage 2 cervical cancer and conferred a 2.51 fold risk of developing cervical cancer at an early stage.	Pandey *et al* (2009)
TLR9	rs5743836 (T1237C)	Cervical cancer	500	Brazilian (Ludwig-McGill cohort)	Most common: hpv16=22.8%; hpv53=15.2%; hpv51=11.4%; hpv84=11.0%	Frequencies not provided TT TC CC	No robust associations were established between the SNP and the risk of HPV infection, nor its clearance or persistence, also per HPV type. The prevalence of the CC genotype was, however, relatively low.	Oliveira *et al* (2013)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
TLR9	2848 G/A (rs382140)	Cervical cancer	120	Chinese Han	HPV16 Cases=64.2% Controls=53%	GG: 97 GA: 2 AA: 1	Association between the SNP and the cervical cancer risk has been observed (P=0.002; OR=7.04, 95% CI=2.02-24.52). The heterozygous genotype was associated with a higher risk of developing cervical cancer in comparison to homozygous genotypes GG or AA (P=0.004, OR=6.92, 95% CI=1.53-33.3; and P=0.036, OR=7.91, 95% CI=0.97-64.52, respectively). A 13.8-fold increase in GA/AA genotype compared with the GG genotype in cervical cancer cases (95% CI=1.761-108.844, P=0.002) has been detected when HPV infection was present. Patients carrying the mutant allele A were also at increased risk for cervical cancer compared with the wild-type G allele (P<0.001, OR=16.026, 95% CI=2.107-121.896).	Lai *et al* (2013)
TLR9	-1486 T/C (rs187084)	Cervical cancer	120	Chinese Han		TT: 99 TC: 0 CC: 1	NS	Lai *et al* (2013)
TLR9	C2848T (rs352140)	Cervical cancer	426	Polish		CC: 27 TC: 51 TT: 22	For the C2848T polymorphism, the adjusted OR for patients with the C/T genotype vs C/C genotype was 1.443 (95 % CI 1.019–2.043, p = 0.0380), the adjusted OR for the T/T genotype vs the C/C genotype was 1.237 (95 % CI 1.016–1.507, p = 0.0328), and the adjusted OR for the T/C or T/T genotype vs	Roszak *et al* (2012)

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
							the C/C genotype was 1.345 (95 % CI 0.976–1.855, p = 0.0700).	
TLR9	-1486 T > C (rs187084)	Cervical cancer	712	Chinese		TT: 40.42 CT: 44.62 CC: 14.97	Heterozygote TC was associated with increased risk of cervical cancer (OR = 1.28, 95% CI = 1.01 – 1.62) compared with TT genotype.	Chen *et al* (2012)
TLR9	-1486 T > C (rs187084)	Cervical cancer	426	Polish		TT: 42 CT: 44 CC: 14	The adjusted OR for patients with the C/T genotype versus T/T genotype was 1.371 (95 % CI 1.021–1.842, p = 0.0361), the adjusted OR for the C/C genotype vs the T/T genotype was 1.300 (95 % CI 1.016–1.507, p = 0.0096), and the adjusted OR for the C/T or C/C genotype vs the T/T genotype was 1.448 (95 % CI 1.099–1.908, p = 0.0083).	Roszak *et al* (2012)

Table 4. *Overview of polymorphisms in genes coding for other categories of proteins (non-cytokine, non-chemokine and non-receptors)*

Gene	Polymorphism	Cohort	*N* cases	Ethnicity	HPV type	Genotype frequency (%) in controls	Result	Reference
Other								
ERAP1 (ARTS-1)	-730	Cervical cancer	75	Dutch		WT/WT: 48.9 WT/MT: 38.7 MT/MT: 13.3	NS	Mehta *et al* (2009)
ERAP1 (ARTS-1)	-528	Cervical cancer	75	Dutch		WT/WT: 61.4 WT/MT: 27.3 MT/MT: 11.4	Genotype distribution significantly associated with presence of lymph node metastases. (p = 0.018)	Mehta *et al* (2009)
ERAP1 (ARTS-1)	-276	Cervical cancer	75	Dutch		WT/WT: 62.5 WT/MT: 31.9 MT/MT: 5.6	Genotype distribution significantly associated with presence of lymph node metastases. (p = 0.017)	Mehta *et al* (2009)
ERAP1 (ARTS-1)	-127	Cervical cancer	75	Dutch		WT/WT: 48.0 WT/MT: 42.7 MT/MT: 9.3	Genotype distribution significantly associated with presence of lymph node metastases. (p = 0.026) Minor allele homozygosity at the ERAP1-127 locus was significantly associated with decreased OS relative to heterozygotes	Mehta *et al* (2009)
ERAP1 (ARTS-1)	-56	Cervical cancer	75	Dutch		WT/WT: 90.6 WT/MT: 9.4 MT/MT: 0.0	Heterozygosity at the ERAP1-56 locus was significantly associated with decreased overall survival (OS)	Mehta *et al* (2009)
ERAP1 (ARTS-1)	Haplotype -127/-56	Cervical cancer	75	Dutch			The occurrence of the minor allele at the ERAP1-56 (c.166G > A) and ERAP1-127 (c.380G > C) loci was associated with decreased survival. Multivariate analysis of the H2 haplotype consisting of a ERAP1-56 major allele and a ERAP1-127 minor allele was performed combined	Mehta *et al* (2009)

							with presence of lymph node metastases and depth of tumour invasion. Heterozygosity (H2 – X) for this haplotype was found to be an independent predictor for OS [HR ¼ 0.219 (95% CI: 0.065–0.731); P ¼ 0.014], as was presence of lymph node metastases [HR ¼ 6.768 (95% CI: 2.182–20.998); P ¼ 0.001].	
CYBA	+11 T > C (rs4673)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
CYBA	-41 G > A (rs1049255)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
CYBA	-16 T > C (rs7195830)	CIN3/Cancer Persistent HPV	470 390	Costa Rican		TT: 9 CT: 43 CC: 48	Increased risk for CIN3 or cervical cancer (P_{trend} value of .04)	Wang *et al* (2009)
FANCA	G501S (rs2239359)	CIN3/Cancer Persistent HPV	470 390	Costa Rican		AA: 38 AG: 48 GG: 14	Increased risk of CIN3 or cervical cancer was found in carriers of this polymorphism (p=0.008)	Wang *et al* (2009)
FANCA	Haplotype G809D (rs7195066) G501S (rs2239359) T266A (rs7190823)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			For A-**G**-G haplotype variant (the one containing the G501S variant), the highest risk for CIN3 and cervical cancer was determined. (OR=1.8, 95% CI (1.4-2.5).	Wang *et al* (2009)
IRF1	-347 G > A (rs839)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IRF1	+11 A > G (rs9282762)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)

IRF3	-81 G > C (rs7251)	CIN3/Cancer Persistent HPV	470 390	Costa Rican		GG: 36 CG: 45 CC: 19	Increased risk for CIN3 or cervical cancer (P_{trend} value of .01) HPV persistence: 1.3-fold risk increase (95% CI, 1.0 –1.7) for CG-genotype and a 1.5-fold risk increase (95% CI, 1.1– 2.1) for the CC genotypes	Wang *et al* (2009)
IRF3	-40 T > G (rs2304205)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
IRF3	+95 A > G (rs2304204)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
JAK3	+291 T > C (rs3008)	CIN3/Cancer Persistent HPV	470 390	Costa Rican			NS	Wang *et al* (2009)
NFKB1	-94 insertion/ deletion ATTG polymorphism (rs28362491)	SCC	233	Chinese Han		$ATTG_1/ATTG_1$: 17.5 $ATTG_1/ATTG_2$: 45.5 $ATTG_2/ATTG_2$: 37.0	ATTG2/ATTG2 genotype and ATTG2 allele in the SCC patients was higher than that of controls, indicating that the 294 insertion/ deletion ATTG polymorphism in NFKB1 promoter was associated with SCC (P = 0.001, OR = 2.560, 95% CI = 1.459–4.492) and (P = 0.001, OR = 1.493, 95% CI 1.168–1.908) respectively.	Zhou *et al* (2010)
OAS1	rs12307655	CIN3/cervical cancer Persistent HPV	416 356	Costa Rican			Associated with progression to cervical precancer/cancer (*P* = 0.0005), associated with HPV persistence (*P* = 0.0034)	Wang *et al* (2010)
OAS2	rs718802	CIN3/cervical cancer Persistent HPV	416 356	Costa Rican			Associated with progression to cervical precancer/cancer (*P* = 0.0001), associated with HPV persistence (*P* = 0.0057)	Wang *et al* (2010)

OAS3	rs12302655	CIN3/cervical cancer Persistent HPV	416 356	Costa Rican			Associated with progression to cervical precancer/cancer (*P* = 0.00008), associated with HPV persistence (*P* = 0.0025)	Wang *et al* (2010)
Pre-miRNA27a	rs895819	Cervical cancer	103	Southern Chinese	hrHPV lrHPV	TT: 53.5 CT: 40.8 CC: 5.8	The T allele was found to be associated with a lower risk of cervical cancer (p=0.036, OR=0.65, 95% CI=0.43-0.97). Individuals with CT + TT genotypes were at a reduced risk of cervical cancer when compared to CC genotype carriers (p<0.01, OR=0.30, 95% CI=0.14-0.66)	Xiong *et al* (2014)
PSMB9 (LMP2)	-60	Cervical cancer	75	Dutch		WT/WT: 45.0 WT/MT: 35.0 MT/MT: 20.0	NS	Mehta *et al* (2009)
PSMB8 (LMP7)	-145	Cervical cancer	75	Dutch		WT/WT: 0.0 WT/MT: 87.3 MT/MT: 12.7	Genotype distribution significantly associated with presence of lymph node metastases. (p = 0.026)	Mehta *et al* (2009)
RNASEL	Arg462Gln (rs486907)	Cervical cancer	98	Argentinian		GG: 35.8 AG: 46.3 AA: 17.9	NS	Barbisan *et al* (2011)
RNASEL	rs3738579	Cervical Cancer	42	Danish	hrHPV	CC: 10.8 TC: 54.5 TT: 34.7	NS	Madsen *et al* (2008)
RNASEL	rs1048260	Cervical Cancer	42	Danish	hrHPV	CC: 48 GC: 40 GG: 12	NS	Madsen *et al* (2008)
RNASEL	rs11072	Cervical Cancer	42	Danish	hrHPV	CC: 48 TC: 40 CC: 11	NS	Madsen *et al* (2008)
RNASEL	rs104825	Cervical Cancer	42	Danish	hrHPV	AA: 48 CA: 40 CC: 9	NS	Madsen *et al* (2008)

RNASEL	rs12135247	Cervical Cancer	42	Danish	hrHPV	AA: 49 GA: 40 GG: 11	NS	Madsen *et al* (2008)
RNASEL	A-T-In5	Cervical Cancer	42	Danish	hrHPV	N/A	NS	Madsen *et al* (2008)
RNASEL	rs12742422	Cervical Cancer	42	Danish	hrHPV	AA: 96 GA: 4 GG: 0	NS	Madsen *et al* (2008)
RNASEL	I97L	Cervical Cancer	42	Danish	hrHPV	AA: 97 CA: 3 CC: 0	NS	Madsen *et al* (2008)
RNASEL	rs6690434	Cervical Cancer	42	Danish	hrHPV	AA: 36 CA: 50 CC: 14	NS	Madsen *et al* (2008)
RNASEL	rs7534480	Cervical Cancer	42	Danish	hrHPV	AA: 14 GA: 50 GG: 36	NS	Madsen *et al* (2008)
SLC11A1 (NRAMP1)	Intron 4	Cervical cancer	127	American		GG: 53.7 CG: 37.0 CC: 9.3	NS	Calhoun *et al* (2002)
SLC11A1 (NRAMP1)	3'-UTR TGTG del	Cervical cancer	127	American		+/+: 96.3 Del/+: 3.7 Del/Del: 0.0	NS	Calhoun *et al* (2002)
SLC11A1 (NRAMP1)	3'-UTR STP+86	Cervical cancer	127	American		AA: 26.9 GA: 62.0 GG: 11.1	NS	Calhoun *et al* (2002)
SMAD7	Exon 4	Cervical cancer	60	Indian			NS	Hariharan *et al* (2009)
SULF1	rs4737999	CIN3/cervical cancer Persistent HPV	416 356	Costa Rican			Associated with progression to cervical precancer/cancer (P = 0.0001), associated with HPV persistence (P = 0.0020)	Wang *et al* (2010)
SULF1	rs4284050	CIN3/cervical cancer	416	Costa Rican			Associated with progression to cervical precancer/cancer (P =	Wang *et al* (2010)

		Persistent HPV	356				0.0007), associated with HPV persistence (*P* = 0.0020)	
SULF1	rs10108002	CIN3/cervical cancer Persistent HPV	416 356	Costa Rican			Associated with progression to cervical precancer/cancer (*P* = 0.0010), associated with HPV persistence (*P* = 0.0181)	Wang *et al* (2010)
TAP1	-333	Cervical cancer	75	Dutch		WT/WT: 60.7 WT/MT: 34.4 MT/MT: 4.9	NS	Mehta *et al* (2009)
TAP1	C/T intron 7	Cervical cancer	200	North Indian		TT: 5.0 CT: 87 CC: 8.0	NS	Kordi Tamandani *et al* (2009)
TAP1	I333V	CIN 2/3	114	New York			Allelic distribution differed significantly between cases and controls (P = 0.02)	Einstein *et al* (2009)
TAP1	D637G	CIN 2/3	114	New York			Allelic distribution differed significantly between cases and controls (P = 0.01)	Einstein *et al* (2009)
TAP2	-665	Cervical cancer	75	Dutch		WT/WT: 7.7 WT/MT: 42.3 MT/MT: 50.0	Heterozygosity and minor allele homozygosity at the TAP2-665 locus were significantly associated with presence of vaso-invasive growth (of the major allele homozygotes, all patients had vaso-invasive growth versus 32% of heterozygotes and 36% of minor allele homozygotes; P ¼ 0.024 and 0.034, respectively).	Mehta *et al* (2009)
TAP2	-651	Cervical cancer	75	Dutch		WT/WT: 89.6 WT/MT: 10.4 MT/MT: 0.0	NS	Mehta *et al* (2009)
TAP2	-565	Cervical cancer	75	Dutch		WT/WT: 84.9 WT/MT: 13.2 MT/MT: 1.9	NS	Mehta *et al* (2009)

TAP2	-379	Cervical cancer	75	Dutch		WT/WT: 72.1 WT/MT: 21.3 MT/MT: 4.9	NS	Mehta *et al* (2009)
TAP2	A/G exon 11	Cervical cancer	200	North Indian		AA: 8.5 GA: 76.0 GG: 15.5	NS	Kordi Tamandani *et al* (2009)
TAP2	I378V	CIN 2/3	114	New York			NS	Einstein *et al* (2009)
TAP2	A665T	CIN 2/3	114	New York			NS	Einstein *et al* (2009)
TAP2	Q685STOP	CIN 2/3	114	New York			NS	Einstein *et al* (2009)
TAP1 *TAP2*	Haplotype rs1057141 (1341) rs1135216 (2254) rs1800454 (1135) rs2228396 (1693) rs241447 (1993)	CIN I/2/3	616	Caucasian (Austrian)		t1341: WT/WT: 75.2 WT/MT: 21.4 MT/MT: 3.4 t2254: WT/WT: 78.2 WT/MT: 18.9 MT/MT: 2.9 t1135: WT/WT: 69.4 WT/MT: 24.8 MT/MT: 5.8 t1693: WT/WT: 89.3 WT/MT: 10.7 MT/MT: 0.0 t1993: WT/WT: 43.7 WT/MT: 44.7 MT/MT: 11.7	The haplotype combination mut-wt-wt-wt-wt (TAP polymorphisms t1135-t1341-t1693-t1993-t2254) was found to be associated with a reduced risk for the presence of CIN (p<0.01 OR 0.5 (0.4–0.8)).	Natter *et al* (2013)

TMC6 (EVER1)	rs9807014 rs3813026 rs11658760 rs383603 rs450474	CIN 3 Cervical cancer	2,767 222	Swedish			NS	Castro *et al* (2012)
TMC8 (EVER2)	rs7208422 rs412611 rs8068430 rs16970849 rs17773842 rs17773854 rs11654773 rs4789015 rs9915090	CIN 3 Cervical cancer	2,767 222	Swedish			rs16970849 (OR $_{AGvsGG}$ = 0.8, 95% CI: 0.66– 0.98, p = 0.03)	Castro *et al* (2012)
TNFAIP8	rs11064 rs3813308	Cervical cancer	1584	Eastern Chinese	Not tested	rs11064 AA 75.3 AG 23.6 GG 1.1 rs3813308 CC 24.4 CG 49.4 GG 26.2	rs11064 genotype GG increases risk of cervical cancer, platinum resistance, and the likelihood of recurrence and death from cervical cancer. The samples used were not tested for HPV.	Shi *et al* (2013)
TNFAIP8L1	rs1060555	Cervical cancer	1584	Eastern Chinese	Not tested	CC 54.9 CG 38.0 GG 7.1	NS	Shi *et al* (2013)

LSIL/HSIL, Low-grade/high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AC, adenocarcinoma; ICC, invasive squamous cell carcinoma of the cervix;
OR, odds ratio; CI, confidence interval; NS, not significant; N/A, not available; WT, wild type; MT, mutant; HR, hazard ratio

[#] Allele frequencies

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PART II

TRANSLATION OF HOST GENOMICS FROM BENCH TO BEDSIDE – A TWO-WAY STREET

CHAPTER 4

Biobanking and translation of human genetics and genomics for infectious diseases

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Abstract

Biobanks are invaluable resources in genomic research of both the infectious diseases and their hosts. This article examines the role of biobanks in basic research of infectious disease genomics, as well as the relevance and applicability of biobanks in the translation of impending knowledge and the clinical uptake of knowledge of infectious diseases. Our research identifies potential fields of interaction between infectious disease genomics and biobanks, in line with global trends in the integration of genome-based knowledge into clinical practice. Furthermore, it examines various networks and biobanks that specialise in infectious diseases (including HIV, HPV and *Chlamydia trachomatis*), as well as examples of successful research and clinical uptake stemming from biobanks. Our article also outlines key issues with respect to data privacy in infectious disease genomics, as well as the utility of adequately designed and maintained electronic health records. We maintain that the public should be able to easily access a clear and detailed outline of regulations and procedures for sample and data utilisation by academic or commercial investigators, and also should be able to understand the precise roles of relevant governing bodies. This would ultimately facilitate uptake by researchers and clinics. As a result of the efforts and resources invested by several networks and consortia, there is an increasing awareness of the prospective uses of biobanks in advancing infectious disease genomic research, diagnostics and their clinical management.

1 Introduction

Over time, the definition of a "biobank" has moved away from an early view of a biobank as population-based to include a wider typology of biobanks that we find in the literature today. In a survey conducted in 2012, researchers involved in managing sample collections were asked about their definition of biobanks. The results of the survey showed the consensus among respondents that the term biobank may be applied to biological collections of human, animal, plant or microbial samples. Additionally, the term biobank should only be applied to sample collections with associated sample data, and to collections that are managed according to professional standards (Hewitt and Watson, 2013).

In post-Human Genome Project research, the role of biobanking as a component of research infrastructure is broadening, as knowledge from biobanks contributed to the understanding of the etiology of multifactorial diseases caused by both mutations in a variety of genes and the influence of environmental factors and lifestyle (Brand and Probst-Hensch, 2007; Knoppers *et al.,* 2012). Furthermore, biobanks have paved the way for the evolution of personalised medicine, especially the development of "tailored" drugs (Gottweis and Zatloukal, 2007). In recent years, integration, analysis and interpretation of data originating from biobanks have begun to play a growing role in our understanding of genetic susceptibilities of infectious diseases. The actuality of infectious diseases and the perpetual challenge they pose for researchers and physicians is reflected in both the high prevalence and the high mortality of existing and growing incidence of emerging infectious diseases (Jones *et al.,* 2008). In fact, infectious diseases represent a major health threat worldwide, and are a particularly significant burden to developing countries (Frodsham and Hill, 2004).

The importance of genetic factors in the pathogenesis of infectious diseases has transformed our understanding of such diseases by incorporating host genetic determinants that modulate immune responses as factors of pathogenesis. We now understand that host responses can determine the outcome of an infection as much as - if not more than - the properties of the pathogen itself (Peng *et al.,* 2009). Genomics outlined molecular biomarkers and pathways as targets for diagnosis or intervention in the field of infectious diseases (Hill, 2006). Relations between genetic factors and susceptibility to the course and the outcome of infectious diseases are predominantly studied through candidate genes, genome wide associations, and twin studies (Hill, 2001). This research means that biobanks (especially large international networks of biobanks driven by the needs of researchers, who require large collections of samples) are an imperative infrastructure for research in host genomics (Meijer *et al.,* 2012).

According to Gotweiss and Zatloukal (Gottweis and Zatloukal, 2007), there are four main types of biobanks:

(1) *clinical case/control biobanks*, which contain biological samples taken from patients with specific diseases and from healthy control patients
(2) *population-based biobanks*, which contain samples from smaller or larger subsets of a population with or without a certain disease;
(3) *population isolate biobanks*, which contain homogenous genetic material of the population represented; and
(4) *twin registries*, which contain samples from monozygotic and dizygotic twins.

Biobanks contain both samples and data; this twofold nature is the root of much of the legal and ethical controversy surrounding biobanks today. Issues concerning privacy health-related information, informed consent, secondary use of samples, and harmonisation of legislation and networking of biobanks are often researched in conjunction with the term "biobanking" (Townend, 2012). The potential impact of biobank-generated knowledge (and its becoming an integral part of public health policies) on our understanding of the etiology of disease, on improving diagnosis and treatment, and ultimately on the health of individuals and populations as a whole has been largely ignored thus far (Knoppers *et al.,* 2012).
Uses of biobanks for public health are (Brand and Probst-Hensch, 2007):
- timely, responsible and effective integration of genome-based health technologies and information into health research, policy and practice;
- supporting the translational process from basic knowledge generated in existing biobanks to the development and implementation of health policies, interventions and programs;
- recognizing the multi-tasking nature of biobanks in the accommodation of different needs by enhancing the ability of biobanks to serve researchers and other relevant stakeholders with particular public health perspectives.
In 1990, Lee identified what he considered to be the ideal properties of a biospecimen bank: a secure, ongoing source of funding; a cryogenic storage facility; selection criteria for obtaining and keeping the best samples in storage; and ensuring the continuation of research to optimize the collection and handling of samples (Lee, 1990). De Paoli (De Paoli, 2005) also identified what he considered to be the main roles of biobanking in microbiology research:
- **to enable unfettered epidemiological research**: prospective use of biobanking is key to detecting and tracking different strains, comparing new strains with previously stored ones, determining modes of transmission, and ultimately combating infections
- to **ensure progress in diagnostics**: by comparing samples taken from the same subjects over time or by comparing samples taken from different subjects at the same point in time, or by applying novel diagnostic tools to the analysis of samples that exhibit increased sensitivity and specificity.

- **to manage studies with large sample sizes**: this may refer to research with sample collections coming from different geographical locations, or research that is conducted in several remote laboratories.
- to **establish biorepositories with characterised host cell lines:** cell lines can be used for research, diagnostics and quality control, and other scientific pursuits.
- **to assist in building a microbial tree of life:** such biobanks provide the basis for mapping out microbial diversity and evolution. Given the increasingly imminent threat that emerging highly virulent or therapy-resistant strains pose for global health, the importance of these types of biobank collections will likely rise in the near future.

In addition to these roles, biobanks can **serve as the foundation for conducting research in host genomics and other 'omic' sciences**, elucidating the role and interactions of the host's immunogenetic factors in infections (Ballana *et al.,* 2012), as well as driving prospective diagnostic and therapeutic advances (Haralambieva and Poland, 2010).

This article examines the role of biobanks in the basic research in infectious disease genomics, and also observes the relevance and applicability of biobanks in both the translation of impending knowledge and the clinical uptake of biobank-generated knowledge in infectious diseases. Our research identifies potential fields of interaction between infectious disease genomics and biobanks, in line with the global trend of integration of genome-based knowledge into clinical practice.

2 Materials and Methods

A literature search was performed on the identification of the existing links between biobanking and infectious diseases; on points of potential collaboration between biobanks, clinics and surveillance agencies; and on the examination of the relevance of electronic health records (EHR) in genomic research of infectious diseases. The study focused on examples of the translation of biobank-generated genome-based knowledge to everyday clinical practice. Databases (PubMed, Cochrane library, Google Scholar), electronic journal collections (Maastricht University EJ collection) and the websites of relevant organizations, networks and consortia (OECD, EAPM, P^3G, BBMRI) were searched for appropriate references. The terms used in the searches were ["biobank*" AND ("infectious disease*" OR "genomic*")]. Retrieved articles were further selected based on relevance. Additional search terms were "public health", "data management" and "data privacy".

This article provides examples of existing biobanks with substantial resources for infectious disease research, such as those for human immunodeficiency virus (HIV), Human papillomavirus (HPV), and *Chlamydia trachomatis* (CT).

3 Results

3.1 Human versus microbial sample biobanks

There is an obvious delineation between samples taken from human individuals suffering from a pathological condition related to an infection (who may or may not be infected with or are carriers of the pathogen) and samples of the infectious agent itself. The former is primarily relevant to host 'omic' research, as it provides the material basis for both candidate gene/SNP-approaches and genome-wide association research in seeking (co)morbidity associations, and also investigates the pathogen interactions with host proteomes (Zhang *et al.,* 2010). These samples should be paired with relevant categories of patient phenotypic data. Pathogen samples enable epidemiological studies, investigate genetic strains of that species, and develop better diagnostic tools and novel therapies (De Paoli, 2005). Biomaterial samples may differ in processing and storage, as well as in shelf-life. As biomedical science moves away from the deconstruction of living systems and turns towards a more integrative, all-encompassing approach (through the likes of systems biology) (Khoury *et al.,* 2007), it is reasonable to expect that researchers increasingly begin to assess host 'omic' data together with infectious agents 'omic' profiles. As a prerequisite, adequate samples should always be accompanied by relevant data.

3.2 Infectious disease genomics and the inflow of data

Advances in sequencing technologies have resulted in a relentless influx of data that need to be interpreted. Currently, researchers are generating data more rapidly than can be analyzed. In particular, the genetic variability of bacteria accumulated through evolution is enormous and significantly increases the volume of datasets. Public Health Genomics specifically emphasises the need to examine all 'omics' (Khoury *et al.,* 2007;Mardis, 2009), which, in the case of infectious disease, involves both the host and the pathogen. This emphasis dramatically increases demand for the effective deciphering of large amounts of data. There are, however, efforts among members of the microbiology community to develop strategies that would make this data manageable. For example, by grouping select loci of the pathogenic bacterial strain into schemes (the so-called *gene-by-gene approach*) and by implementing themes schemes in conjunction with conventional (sequence-based) schemes in adequate database platforms, data that is obtained through different studies and can be used more effectively in combined analyses (Maiden *et al.,* 2013). In this way, "genotype summaries" of selected genes could be linked to phylogenetic relationships and functional characteristics of bacteria, thereby helping researchers navigate vast bacterial genomic diversity.

3.3 Existing infectious disease biobanks and networks

The number of laboratories that are creating their own biobanks is difficult to quantify with full precision. However, the number of organisations that are developing nation-wide or transnational collections and are building large consortia and networks is ever increasing (De Paoli, 2005). Large networks such as the Public Population Project in Genomics and Society (P^3G) (http://p3g.org/) and Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) (http://bbmri.eu/) are changing the landscape of international collaboration in biobanking, harmonizing regulatory legislation and thus facilitating the use of biobanks in research (Wichmann *et al.,* 2011). Promising nation-wide models have also emerged, including the United Kingdom Biobank (http://www.ukbiobank.ac.uk/), the Swedish National Biobank programme (now the Swedish arm of BBMRI, http://www.bbmri.se/), the Iceland Biobank, and others. Some biobanks, however, are not positioned as public domain entities; in the case of Iceland, for example, the biobank is a private company that has been given a commercial data license (Mitchell, 2010).

This article presents several infectious disease biobanks and their effective usage, which has led to cases of successful clinical uptake (or its near-future prospects) for HIV, CT and HPV (for which there is a more substantial body of literature available). These biobanks are founded and governed by hospitals and academic institutions and provide clear examples of how biobanking can stimulate research in infectious disease genomics.

3.3.1 HIV

The Infectious Diseases Biobank (IDB) at King's College London is an oft-cited example of an infectious disease-oriented biobank (Kozlakidis *et al.,* 2012; Towie, 2007; Williams *et al.,* 2009). The IDB is actively collecting samples from patients infected with HIV, hepatitis B and C viruses, and invasive bacteraemias (such as the methicillin resistant *Staphylococcus aureus* (MRSA)). The IDB is also collecting samples from healthy control subjects. The number of HIV patients with archived materials in the IDB is steadily increasing, resulting in the availability of distinct patient cohorts (in meaningful numbers) to researchers. Data on the IDB's website indicate that by September 2010, HIV sample donations had reached 500 annually. Examples of research stemming from this collection are (as stated on the IDB website): the roles of vpu gene and tetherin in HIV/AIDS pathogenesis; gene expression signatures in *in vivo* and *in vitro* HIV-1 infection; non-infectious HIV co-morbidities; renal function and bone homeostasis in patients on HAART; the definition of CD161+ CD8+ T cell subset function in HIV infection and their response to therapy; the effect of Maraviroc on microbial translocation in HIV infected individuals

receiving antiretroviral therapy; and the metabolic impact of Darunavir/ritonavir maintenance monotherapy after successful viral suppression with standard Atripla in HIV-1-infected patients. Aside from archiving biological samples, the BioBank runs a database in which the following clinical information on HIV donors is archived: histories of CD4+ cell numbers and plasma viral loads; last known HIV negative result and first positive HIV test; birth date; ethnicity; gender; HAART administering; and other complications. The database also features sample processing information (dates and times of venepuncture, processing and freezing); and details on aliquots that have been stored or transferred to researchers.
Another example of an infectious disease-oriented biobank is the Spanish HIV BioBank (Garcia-Merino *et al.,* 2009). The primary objective of this biobank is to further scientific knowledge about HIV infection by providing biological samples from HIV-infected patients that are included in cohorts for the objective of carrying out research. The HIV BioBank receives samples from 28 hospitals, spread across Spain, which are grouped into 6 cohorts of HIV-patients, each with defined characteristics. Any member of the AIDS Network, or any party to a relevant collaboration with a member can apply for samples. Sample release applications are evaluated by members of the Scientific Committee. If the project is approved, the researcher signs a Release Agreement with the director of the BioBank and with the coordinator of the Cohort. The BioBank and the Cohort are responsible for locating the type and number of samples needed to carry out the project. Once a year, after the samples have been released, the principal researcher sends a scientific report to the BioBank containing his or her results, such that the BioBank can maintain up-to-date records on all projects.

3.3.2 Chlamydia trachomatis

Partners of the EpiGenChlamydia Consortium (urogenital and ocular CT infections), coordinated by the London School of Hygiene and Tropical Medicine (by David Mabey and Robin Bailey and their Gambian partners) who are researching ocular Chlamydia-related conditions, have already defined and secured 1500 case-control pairs (n=3000). More than 4000 specimens that are currently in use have been collected by Dutch partners, and 10000 specimens are available for further studies (Morre *et al.,* 2009). One goal was to build a biobank and data warehouse – a biomedical, ethically-developed and run central sample collection and data management system. The Consortium is investigating possibilities for conducting genetic and epidemiologic Chlamydia research with samples from existing biobanks in Northern European countries.
The Consortium also aims to structure trans-national research to such a degree that comparative genomics and genetic epidemiology can be performed on large numbers of unrelated individuals. The most pivotal deliverables of this project were biobanking and data-warehouse building. These deliverables will allow for continuous generation of

scientific knowledge on CT-host interaction genetic predisposition to CT infection, and the development of tools for early detection of this predisposition. The study of sequence variation (mainly SNPs) is a technique employed by different consortium partners to gain insight into the differences in clinical courses of infection, in order to identify genetic markers for susceptibility.

A review by Malogajski et al. (Malogajski *et al.,* 2013) gives an overview of immunogenetic factors that have a demonstrable effect on human susceptibility to and the severity of CT. These immunogenetic factors are alleles (determined by specific SNPs) of pathogen recognition receptor genes. Women carrying one or a combination of these alleles are at higher risk of contracting CT, or are at significantly higher risk of developing subfertility-related complications, such as tubal pathology. The review proposes the development of novel diagnostic tools for assessing individual risk faced by CT-positive women. Currently, clinicians employ CT IgG serology when assessing these risks (Broeze *et al.,* 2011). Due to limited sensitivity and specificity of CT serology, the predictive value of this serology is weak, and as a result, many physicians recommend that women undergo additional invasive, stressful, and costly diagnostic procedures. It is estimated that 40-45% of women undergoing laparoscopy do not have tubal pathology. Additionally, false negative serology results account for about 20% of women whose tubal pathology will not be properly and timely diagnosed (Lal *et al.,* 2013). The proposed tool would introduce a diagnostic approach based on a combination of two factors: a predictive SNP load; and serological markers for CT infection. On-going research aims to validate this set of SNPs and a subsequent cut-off score for diagnostic purpose. This tool would be the first to use a genetic trait in the diagnosis of infectious diseases severity in the triage of women.

3.3.3 HPV

Similar to the translation of CT biobank-derived data into diagnostic applications for subfertility, the translation of HPV research results should contribute to better diagnostics of cervical cancer and its pre-neoplastic stages, cervical intraepithelial neoplasia (Malogajski *et al.,* 2013). Large biobanks and patient cohorts are used to achieve this result. Despite the anticipated outcomes of HPV vaccination (which should lead to a drop in cervical cancer incidence in a matter of decades), the needs of generations of women who were above the age of expected exposure to HPV virus (and were therefore left out of vaccination programs) ought to be addressed. The cervical scraping cytomorphology assessment, better known as the PAP test, is routinely used throughout the world as a screening tool for cervical lesions; however, the PAP has low sensitivity. The introduction of high-risk HPV (hrHPV) assessment will increase sensitivity and ease (especially in the case of self-collected vaginal swabs) to determine a woman's risk of developing cervical cancer. Referral to a gynecologist is only needed where a woman is found to be infected

by a hrHPV type. Some authors propose the development of a triage tool for high-risk HPV positive women based on methylation markers; this means that a woman should only be referred for further examination if she tested positive for one or more hrHPV types and at the same time carries a combination of methylation markers indicative of a progression of pre-neoplastic stages. This approach builds upon a number of studies that show how epigenetic alterations become increasingly present with each successive stage of cervical lesion and cervical cancer, mainly in genes that are important to the progression of cancer (e.g. tumour suppressors and cell adhesion molecules), or genes coding microRNAs, whose role is to bind to the viral nucleic sequences, thereby making them inaccessible to enzymes that are replicating or transcribing. Based on the available studies at that time, the review (Malogajski *et al.,* 2013) highlighted methylation patterns in MAL and CADM1 genes as optimal markers for the development of a potential triage test (Overmeer *et al.,* 2011). A more recent review by Litjens et al. (Litjens *et al.,* 2013) reached the same conclusion, but added $p16^{INK-4a}$/Ki-67 dual immunostaining and viral integration to the proposed set of markers.

3.4 Electronic health records

The rise in usage – and usability – of electronic health records (EHRs) is a demonstrably promising catalyst in the efforts to better utilise and standardise biobanks (Kohane, 2011). This pertains to handling the information on the biomaterial from the large cohort studies on a plethora of diseases, including infectious diseases. 'EHR-driven genomic research' can ideally be achieved using two distinct workflows. Firstly, patients with a particular infectious disease or related sequelae could be selected from the EHR by using language processing tools, such as natural language processing (NLP) tools. In this case, the selected population could thereupon be recruited, either for the purpose of providing samples for genomic research, or in order to verify whether residual samples taken on previous occasions could be utilised. Secondly, EHRs can be used to broaden and advance clinical characterisation by adding new relevant data to the files of those individuals whose samples are already stored in another biobank or have been used in the context of a cohort study (Kohane, 2011). Electronic systems for the automated detection of notifiable diseases have, in fact, been tested using EHRs. In past decades, the term of preference was electronic medical records (EMRs). This term has gradually been overtaken by the previously mentioned EHRs, as focus slowly expanded from the inclusion of basic clinical patient data to the provision of a more complete insight into their health background and care.

The so-called ESP (Electronic Medical Record Support for Public Health) algorithmic system, which has been tested on Chlamydia records and others, assists not only in the

identification and reporting of cases of notifiable disease, but also in the advancement of public health. Prospective applications of EHRs include syndromic surveillance; clinical decision support; the construction of vaccine registries; and the assessment of areas with higher prevalence of disease (Klompas *et al.,* 2007). The incorporation of patient genome-based information into EHRs would undoubtedly act as a major driving force for genomic medicine. It would enable the investigation of potential comorbidities of genomic associations (Kohane, 2011), and would elucidate the ways in which such associations can individually or synergistically result in increased susceptibility to or severity of infectious disease. That said, the incorporation of patient genome information into EHRs has thus far been a daunting task, since most EHR systems are not designed to include genomic data (Kullo *et al.,* 2013). Although the linear DNA sequence is simple by nature, the sheer volume of data and the complexity of relations among the 'functional components' of DNA are significant hurdles in attempting to devise an EHR system using this information (Kullo *et al.,* 2013; Masys *et al.,* 2012).

3.5 Data privacy and infectious disease genomics

While the issues of data privacy and consent exceed the parameters of this article, we will briefly lay out the state of the art in this field, as well as how legal frameworks, governance of infectious disease biobanks and the handling of sensitive data can affect not only patient rights, but also biomedical research generally. In 2013, during the Irish presidency of the EU Council, the European Alliance for Personalised Medicine (EAPM) hosted a conference on innovation and patient access to personalised medicine, in which experts discussed recent advances in healthcare and formulated conclusions relating to these advances (EAPM, 2013). In order to optimise data security and to facilitate access and consent (which would allow for re-use and secondary use of data), it was concluded that robust legal regulation of personal data in scientific research should be implemented. Moreover, cross-border transfers of data for the purposes of scientific research should be stimulated in cases where such privacy instruments have been deployed. It is important to note that, in harmonizing different systems of governance, a balance must always be struck between the stimulation of cross-border transfers of data and individual rights to privacy.

The importance of data protection cannot be underestimated, especially in the handling of samples taken from individuals who are afflicted with serious infections. Genomic discoveries concerning such infections potentially create various forms of discrimination in the context of future discovery. For example, it was discovered that African-American carriers of a polymorphism conferring a Duffy antigen-negative phenotype, DARC -46C/C, are resistant to malaria (*Plasmodium vivax* infection). However, subsequent research into this polymorphism also revealed that carriers have a 40% increased likelihood of becoming

infected with the HIV-1 virus (He *et al.,* 2008). In this case, contrary to the protective character of CCR5- Δ32 deletion as witnessed in European populations, the disruption of the expression of a functional receptor is a major disadvantage to the carrier. Evidently, the risks of stigmatisation and discrimination arising from genome-based information (the disclosure of a patient's illness or infection status being a potential infringement of patient rights) cannot be ignored. As stated in the EAPM report conclusions (EAPM, 2013), progress must be achieved by developing trust between researchers and the public, and by promoting the equal treatment of health research data (including genome-based information included) and the removal of silos for single-use data. Since this information is theoretically unlimited in terms of longevity, robust data protection mechanisms must be in place for periods longer than the samples' shelf life (Heeney *et al.,* 2011).

At the same time, different sets of mechanisms are needed in parallel with vigilant data protection. Genome-based research necessitates large sample sizes in order to arrive at more reliable results; as a result, overly-restrictive data protection policies can impede research and innovation (Masys *et al.,* 2012). A large number of samples is necessary to identify patient subgroups of interest. There are also calls for the provision of research data to the general public, particularly in cases where the research itself was funded using taxpayer dollars (Church *et al.,* 2009). Entire human genome sequences, including those of several prominent researchers, have already been made available to the general public online (open access model). In spite of this open access, the debate over balancing the right to consent versus the right to privacy is far from resolved. The fact that fewer than 13-15 genomic locations with variable repeats (or 30-80 statistically independent SNPs) can be used to identify any one individual (Lin *et al.,* 2004) lends perspective to requests for the deregulation of data sharing. Samples that contain a 'genomic fingerprint' in combination with data relating to the presence of serious infections pose a new threat to those safeguards that ensure participant anonymity and prevent partial treatment. Due to lack of funding, many academic institutions allow private organisations to handle their genomic databases; as a result, the protection of the rights of human participants may be at risk in any future commercial uses of data (De Paoli, 2005).

4 Discussion

The aim of this review was to explore empirical evidence on the role of biobanking in infectious disease genomics and to outline the pertinent issues in setting up and utilizing biobank materials. We note that published material that provides a detailed overview of existing infectious disease biobanks and their uses to date is lacking. In order to facilitate extensive collaboration with researchers and to ensure the continuation of research on infection, infectious disease biobanks must become more visible, and must emphasise their societal impact. Thus far, the authors have encountered underrepresentation of infectious disease biobanks in publications and insufficient information on official websites. The public should be able to easily access a clear, detailed outline of regulations and procedures for sample and data utilisation by academic or commercial investigators, as well as an account of the precise roles of governing bodies. Examples of procedural transparency and extensive online visibility include the King's College Infectious Disease BioBank and the Spanish HIV BioBank (Garcia-Merino *et al.,* 2009; Williams *et al.,* 2009). Appropriate regulation should precede the effective translation of biobank-based research to clinical settings; such regulation necessitates intensive efforts, so as to ensure rapid clinical uptake.

In recent years, several biobanking consortia and extensive networks have been formed, and there has been a visible increase in efforts, stakeholders' involvement and resources allocated (Wichmann *et al.,* 2011). Nevertheless, infectious disease biobanks have yet to achieve their full potential. This review does, however, provide several examples of biobanks that have successfully contributed to the translation of data to clinics and patients. In order to successfully utilise biobank information in research on infectious disease, and in order to develop 'tailored' therapies based on pharmacogenomics research, adequate representation of ethnic minorities and neglected populations in biobanking is of paramount importance. Biobanks must be constructed to account for ethnic differences in susceptibility to certain infectious diseases, which themselves have been extensively documented (Dolo *et al.,* 2005; Velez *et al.,* 2010). In HIV-AIDS therapy research, for example, extrapolations of potential clinical implications of allele frequency differences between different ethnicities could significantly assist doctors when prescribing therapies. Consortium for the BioBank and Pharmacogenetics database of African populations is an example of efforts paving the way for individualised treatments for HIV-AIDS (Matimba *et al.,* 2008). The Consortium's biobank of anonymous samples was used to determine baseline frequency distribution of SNPs of genes affecting drug metabolism; this usage enabled the establishment of a pharmacogenetics database. Certain information can be essential for optimizing therapeutic approaches and reducing ethnic-specific adverse reactions, such as the different drug-metabolizing capacities of particular allelic versions of enzymes (such as the CYP2B6*6 allele) (Matimba *et al.,* 2008).

There is an argument to be made, however, that these differences are neither inherent nor applicable to all infections. Some authors argue, in terms of decreased precision of data analysis, against blind 'social inclusivity' in biobank sampling at the *potential* expense of 'analytical acuity.' (Smart *et al.,* 2008). In countries such as the UK or the US, acting more fervently upon the two aforementioned views could lead to a reevaluation of the manner in which biobanks are governed.

EHR system designers need to be encouraged to configure these systems so as to enable the incorporation of genome-based (or 'omic'-based) information. The addition of pathogen 'omic' data to an accompanying registry should be made feasible in order to promote research in infectious diseases. Other forms of research, the clinical uptake of genome-based knowledge, and the advancement of personalised medicine can all invaluably benefit from a shifting approach to health record management. Several approaches have been proposed to this effect, and each acknowledges the unique nature of genomic data (Jing *et al.,* 2012; Masys *et al.,* 2012).

The unique challenges associated with biobank-based research indicate that it is in some aspects more complex than other types of health or biomedical research. One of the main obstacles to translating biobank data into the clinical setting is confidentiality and privacy, which stem from a necessary pairing of biobank information with personal and unrelated types of health information. The protection of data obtained from samples of patients who are afflicted with serious infections is of particular importance due to the potential in such cases for discrimination. Discrimination can result both from current interpretations of data, and from future research and upcoming innovations in genomic technologies.

5 Conclusions

A clear overview of the usage of existing infectious disease biobanks is lacking in present literature, and we maintain that this information should be readily accessible to the public, along with clear regulatory and procedural guidelines for utilisation of samples and data. This would ultimately facilitate the currently insufficient uptake by researchers and clinics. Several biobanks have, however, already set high standards in terms of instating appropriate regulation as well as enabling successful translation into clinical setting and can therefore serve as a model to other biobanks. In recent years, efforts and resources that have been invested in biobanking networks and consortia have surged. As a result, there is a higher awareness of the multitude of ways in which biobanking can advance basic research, diagnostics and - most importantly – the clinical management of infectious disease. These advances will ensure that research in biobank-based infectious disease continues to progress.

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CHAPTER 5

Translational potential into health care of basic genomic and genetic findings for human immunodeficiency virus, *Chlamydia trachomatis* and human papillomavirus

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Abstract

Individual variations in susceptibility to an infection as well as in the clinical course of the infection can be explained by pathogen's virulence factors, environmental factors, and host genetic differences. In this paper we review the state-of-the-art basic host genomic and genetic findings' translational potential of human immunodeficiency virus (HIV), *Chlamydia trachomatis*, and human papillomavirus (HPV) into applications in public health, especially in diagnosis, treatment, and prevention of complications of these infectious diseases. There is a significant amount of knowledge about genetic variants having a positive or negative influence on the course and outcome of HIV infection. In the field of *C. trachomatis*, genomic advances hold the prospect of a more accurate subfertility prediction test based on single nucleotide polymorphisms (SNPs). In HPV research, recent developments in early diagnosis of infection-induced cervical cancer are based on methylation tests. Indeed, triage based on methylation markers might be a step forward in a more effective stratification of women at risk for cervical cancer. Our review found an imbalance between the number of host genetic variants with a role in modulating the immune response and the number of practical genomic applications developed thanks to this knowledge.

1. Introduction

Infectious diseases represent a major health threat worldwide and a significant part of the burden of disease in developing countries (Frodsham & Hill, 2004). Public health policy has traditionally had an important role in tackling such threat through established measures of prevention, mostly by controlling social and environmental determinants of health and through vaccination. With the recent advances in Public Health Genomics, public health moved its focus from a "one size fits all" approach in health promotion and prevention activities to targeting populations and subpopulations with defined genetic risks and developed its unique role, translation of genome-based knowledge and technologies into public health policy and practice, and its integration across disciplines (Borke *et al.,* 2006). Scientific developments in basic research and the development of Public Health Genomics have changed many paradigms regarding infectious diseases. Indeed, the recent evidence of genetic factors in the pathogenesis of infectious diseases transformed the view of such diseases from strictly pathogen-centric to the one incorporating host genetic determinants that modulate immune response. Though research in the field of genetic susceptibility to infectious diseases started in 1954, recent progress in genomics led to the characterisation of molecular biomarkers and pathways as understanding of infectious diseases explains the individual variation in susceptibility to an infection as well as the clinical course of the infection by pathogen related factors, environmental factors, and genetic differences. The field identifies genes responsible for influencing susceptibility to infections as well as their severity and response to treatment. This is predominantly achieved by studying candidate genes, genome wide associations, and twin studies (Hill, 2001). A great amount of effort and resources have been directed to obtaining knowledge about host genetic components of infectious diseases and to confirm associations in order to develop genomic applications in everyday clinical practice and prevention. Nonetheless, although the amount of genetic data in relation to disease is increasing exponentially (Khoury *et al.,* 2011; Rowell *et al.,* 2012), there is a clear lack of translation of such findings to healthcare applications. Indeed, the amount of information about basic genome-based scientific findings present in the scientific journals is disproportionate to the number of patents and marketed products used in hospitals (Lal *et al.,* 2011). In this paper, priority was given to three sexually transmitted diseases of significant public health relevance: HIV, HPV, and *Chlamydia trachomatis* genital tract infections. The aim of this review is to provide a state-of-the-art overview on the translational potential of basic genomic and genetic findings related to HIV, *C. trachomatis*, and HPV infections, into applications in public health focusing on their diagnostics and treatment.

2. Methods

Based on our field of expertise in sexually transmitted infections (STIs), we selected the most prevalent bacterial STI, *C. trachomatis,* and the two most prevalent viral STIs, human papillomavirus (HPV) and the human immunodeficiency virus (HIV), knowing that for these infectious diseases human genetic and genomic markers are described in the literature.
We used the HuGE Navigator Version 2.0: an integrated, searchable knowledge base of genetic associations and human genome epidemiology (http://hugenavigator.net/) (Yu *et al.,* 2008) to identify papers with a description of potential translation on the basic findings of genetic and genomic markers into diagnostic applications and ultimately into public health. Identified papers and authors were expanded using PubMed searches. For each infectious disease a general introduction will be given, the key genetic and genomic markers will be described, and the translational potential outlined. Finally, a general discussion and conclusions will be provided.

3. Results

3.1. HIV

Despite the decrease in incidence of HIV infection (in 2009 the number of newly infected individuals dropped by almost 20% compared to the previous year), the prevalence of HIV is still very high. At the end of 2009, it was estimated that there were 33.3 million people living with HIV. The growing prevalence and the reduction in the AIDS-related mortality are mainly attributed to the success of antiviral therapy (UNAIDS, 2010). Nonetheless, the public health relevance of the disease remains indisputable, as tackling HIV requires large financial expenditures, and it is still among the sexually transmitted diseases causing the highest morbidity and mortality and it is highly preventable (CDC, 2001). As mentioned earlier, research in the field of infectious diseases has established that the susceptibility of an individual is also modulated by host genomic factors. In this context, recent genomic and genetic discoveries using candidate gene and genome wide association studies (GWAS) increased our knowledge of the association among genetic loci from the so-called "major susceptibility genes." HIV infection is the most studied infection by the aforementioned approaches. The research of a genetic role for the individual differences in the course of infection, besides offering new strategies for developing a treatment or a vaccine, also provides basic insights in the immunopathology of the infection. Moreover, this newly collected evidence could provide an opportunity of identifying persons at higher risk of getting or progression of the infection. On the other hand, this could detect patients having genes that make them long-term non-progressors, thus with delayed or no progression to AIDS.

3.1.1. Review of the host genetic variants found to influence HIV infection

The review of papers written by the experts in the field of host genomic determinants of infection, disease progression, and disease outcome reveals the growing body of host genomic biomarkers by the year. However, few associations were positively confirmed. Among these, only 15–20% of observed genetic variants have been identified as influencing HIV infection (An & Winkler, 2010). Many studies and reviews place genetic variants of chemokine receptor and chemokine ligand genes, HLA and related genes on top of the list of influential genetic factors identified in HIV infection (O'Brien *et al.,* 2000; Tang & Kaslow, 2003; Kaslow *et al.,* 2005; An & Winkler, 2010; He *et al.,* 2010). Chemokine receptors have an important role in modulating HIV-1 early infection. Particular attention has been given to *CCR5* and *CCR2* genes, encoding co-receptors on the surface of the CD4+ lymphocytes, crucial for HIV cell entry. In the initial stages of the infection, the HIV virus uses CCR5 as a preferred co-receptor (Kaslow *et al.,* 2005). As a result, a mutation in the chemokine receptor genes resulting in the absence or significant reduction of CCR5 molecules on the cell surface would have a protective effect. Indeed, the expression level of this co-receptor influences the HIV infection outcome, and mutation of this molecule is associated with the ability of the virus to enter the cells in vitro, the in vivo viral load, the CD4+ levels during highly active anti-retroviral therapy (HAART: combination of three or more antiviral drugs), and the progression of the diseases to AIDS.

In 1996, it was discovered that the deletion of 32 base pairs of *CCR5* (*CCR5*Δ32) results in shortened and inactive proteins. So far, *CCR5*Δ32 remains the only discovered mutation that completely protects homozygotes from HIV infection and in heterozygotes slows down the progression of the disease (An & Winkler, 2010). Moreover, the discovery of *CCR5*Δ32 genetic variant opened the door for the development of a new type of anti-HIV medications. Data obtained from *CCR5* gene candidate studies have been rather timely applied in the pharmaceutical industry, leading to the development of novel therapies, as further discussed in the next section. In addition, the association between the +190 A>G mutation of CCR2 chemokine receptor and the delayed onset of AIDS was discovered in 1997. The resulting substitution of the amino acid valine, at the position 64 of *CCR2*, to isoleucine influences HIV progression, but not the risk of HIV infection. HIV positive patients carrying this mutation showed delayed progression to AIDS by two to four years (Smith *et al.,* 1997).

3.1.2. Application of Research Based on Chemokine Receptors

As stressed earlier, the major goal of the research on host immunogenetics of HIV is to acquire knowledge of how differences in genetic variants are influencing individual susceptibility to infection and developing new drugs based on that. The research provided insights into the effects of CCR5 co-receptor blockade and down-regulation on HIV infection (Hütter & Ganepola, 2011). As a result drugs with a new mechanism of action, the blockage of CCR5 receptors, were developed. These drugs are also known as entry inhibitors. So far there are only two approved such drugs in clinical use, Maraviroc (Pfizer) and Enfuvirtide (Roche) (McKinnell & Saag, 2009; Singh & Chauthe, 2011). Of the two, Enfuvirtide was the first to be FDA approved. The success of this drug, despite its proven antiviral efficacy in patients' treatment, was constrained by the difficulties related to its subcutaneous administration, causing skin abscesses. The first orally administered HIV entry inhibitor was Maraviroc, approved by the FDA for patients with R5 virus types in 2006. The drug binds to the CCR5 chemokine receptor causing a conformational change that blocks the gp41-mediated fusion of viral and cellular membranes (McKinnell & Saag, 2009). The next most promising HIV entry inhibitor is Vicriviroc (Schering-Plough), amedicine with the same action mechanism as Maraviroc, but expected to be more effective. Vicriviroc has still not been approved by FDA, but phase III clinical trials have been recently completed (Gilliam *et al.,* 2011). A recent extensive review of HIV-1 entry inhibitors patented from 2004–2010 (Singh & Chauthe, 2011) revealed 35 small CCR5 antagonist molecules patented by five different pharmaceutical companies (Astra Zeneca, ViroChem Pharma, Anormed, Inc./Genzyme Corp., Euroscreen, and Ono Pharmaceuticals). In the same review, it was found that the number of patents for CXCR4 (co-receptors for X4 HIV strains) antagonists and dual CCR5/CXCR4 antagonists is significantly lower. Further, clinical developments of CXR4 antagonists have been delayed in preclinical and clinical studies due to serious side effects (cardiac abnormalities and liver toxicity) or lack of drug efficacy. Human Leukocytes Antigen (HLA) genes encode proteins that present antigens to T and B lymphocytes. There are two classes of HLA genes: class I (loci A, B, and C) and class II genes. A strong association has been observed between HLA I alleles and protection/susceptibility to HIV (Carrington & O'Brien, 2003). The effect of HLA A, B, and C homozygosis in general is accelerated AIDS progression. Other confirmed associations include *HLA* alleles B*27 and B*57 and delayed progression to AIDS (Carrington & O'Brien, 2003; den Uyl *et al.,* 2004; Kaslow *et al.,* 2005; Singh & Spector, 2009). On the other hand, the B*35 allele is associated with increased susceptibility and more rapid progression of the disease. The median time in which homozygous carriers of the B*35 allele develop AIDS is half the time of non-carriers of such alleles (O'Brien & Nelson, 2004).

The association between genetic variants of HLA class I loci and *CCR5* and the pathogenesis of HIV infection has been confirmed in recent years by many GWAS studies. However, GWAS did not identify further major susceptibility loci (Chapman & Hill, 2012). Association studies between HLA class II alleles and the susceptibility to the HIV infection has been less consistent. HLA genes have also been shown to have a role in the Mother to Child Transmission (MTCT) of HIV infection. Indeed, HLA class I concordance between mother and child is associated with higher risk of transmission, vice versa HLA discordance is associated with a lower risk (Singh & Spector, 2009).

3.1.3. Application of Research on HLA Genes

Although none of the mentioned HLA genes have yet been identified as a target for new drugs, the information gathered on the disease progression modulated by different genotypes has provided valuable information for clinical trials (Carrington & O'Brien, 2003). Research on HLA alleles led to important pharmacogenetic applications. *HLA* B*5701 positive patients, who are at risk for hypersensitivity to Abacavir (a nucleoside reverse transcriptase inhibitor), cannot be treated with this drug. This serious, and possibly fatal, adverse drug reaction is present in 5% of patients (Mallal *et al.,* 2002). Genetic testing of all the individuals before prescribing the drug prevents serious side effects, building a very strong case for a stratified medicine approach, tailored to individual genetic characteristics. The idea behind it is that our personal genetic differences create a need for accordingly different treatment approaches. In the case of Abacavir recognizing interpersonal variation in reaction to drug is an excellent example of stratifying HIV treatment based on genetic research. In summary, HIV immunogenetic research provided some basic insights into the immunopathology of the infection and gave foundations to the development of new drugs for the therapy of the infection. Ideally this will be just the first step in advancing therapies. Information on individual susceptibility, higher or lower individual risks, and delayed or accelerated AIDS progression associated with certain gene variants will make a more individually tailored treatment possible in the future.

3.2. Chlamydia trachomatis

C. trachomatis is a leading cause for a variety of diseases including ocular, respiratory, and sexually transmitted diseases. This section of the review will only focus on the latter, since sexually transmitted *Chlamydia* infections are the most common worldwide, whereas, for instance, ocular infections are mostly seen in third world countries. Host genetic twin studies of ocular *C. trachomatis* have shown that 40% of the responses to the pathogen are dependent on host genetics (Bailey *et al.,* 2009). According to the WHO, "more cases

of STI are caused by *C. trachomatis* than by any other bacterial pathogen" (WHO, 2012). The persisting high incidence of roughly 100 million cases per year worldwide makes *C. trachomatis* infection an enormous health problem throughout the world. The bacteria can be easily eliminated by antibiotic treatment; however, as a result of often being asymptomatic, the infection is frequently diagnosed too late or not at all. Infertility, premature delivery, PID, and ectopic pregnancy are among the serious sequelae of the untreated infection (Starnbach & Roan, 2008). Evaluation of the causal link between *Chlamydia* lower genital tract infection and tubal infertility is very challenging due to the fact that this is a "silent" complication, usually diagnosed years after the infection (Land *et al.,* 2009). Infected women can either clear the bacteria without any damage to their reproductive functions or develop severe late complications, such as tubal occlusion and periadnexal adhesions, leading to infertility as the most severe of complications. The differences in disease outcome are often determined by genetic variations, such as single nucleotide polymorphisms (SNPs) in genes responsible for, amongst others, bacterial sensing receptors (and the pathways to which they belong) on cells such as macrophages as well as local vaginal and tubal epithelial cells. The higher the number of genes affected by SNPs, the more abnormal the immune response, leading to a higher chance of severe complications (den Hartog *et al.,* 2006b). Inadequate recognition of the pathogen and consequent inadequate immune response lead to a higher risk of subfertility (den Hartog *et al.,* 2006a). In a research performed on Gambian twins (Bailey *et al.,* 2009), it was estimated that 40% of variation in *Chlamydia* infection characteristics could be explained by differences in host genetic factors.

3.2.1. Review of the Host Genetic Variants Found to Influence Chlamydia Lower Genital Tract Infection

TLR Receptors

Toll-like receptors (TLRs), with their role in identifying pathogens and initiating innate immune response, have been recognised as the most important factors in influencing differences in susceptibility to course and outcome of Chlamydia infection (Darville *et al.,* 2003; den Hartog *et al.,* 2009). Indeed, much of immunogenetic research in this field is focused on *TLR* genes and genes involved in their pathways, not only by mRNA- and protein-based studies but also by studying the association between SNPs in *TLR* genes leading to the loss of function of the receptors and the potential higher risk of late complications such as tubal infertility. The application of such research could be in the area of early diagnosis of tubal infertility or subfertility. Based on this evidence, the time now being lost as a result of late or misdiagnosis of tubal infertility could be directed to IVF attempts. So far, there are ten TLRs identified in humans, recognising different

bacterial and viral components. TLRs activate signaling pathways of immune response against different pathogens by activating different inflammatory cytokines (Kawai & Akira, 2006). TLR2, TLR4, and TLR9 recognise pathogen-associated molecular patterns (PAMPs) of *C. trachomatis*. Genes for TLR receptors 2 and 4 are considered particularly important in modulating innate immune response to *C. trachomatis* (Laisk *et al.,* 2010). Several studies showed that SNPs in *TLR4* have a role in making women more prone to subfertility as a late complication of Chlamydial infection. Nonetheless, the exact role of TLR4 in subfertility has not been yet clearly understood (Darville *et al.,* 2003; den Hartog *et al.,* 2009). Subfertile women who have IgG antibodies for *C. trachomatis* have a two times higher likelihood to be carriers of the *TLR4* +896 A allele, compared to women without tubal pathology (den Hartog *et al.,* 2009). Although this observation was not statistically significant, reported trends suggest that it could be worthwhile to further explore it in a larger cohort. Further, murine studies showed that TLR4 functional mice are more protected against reinfection compared with mice with dysfunctional or absent TLR4 (Laisk *et al.,* 2010). In their study of genetic variants involved in the immune response regulation in genetic tract infections, Laisk et al. found that the *TLR4* +896 A>G and +1196 C>T polymorphisms protect against multiple infections with *C. trachomatis, N. gonorrhoeae, M. hominis, M. genitalium, U. parvum, and U. urealyticum*.

Depending on the patient definition (i.e., including or excluding *C. trachomatis* serology), they found that specific *MBL2* high producing haplotypes can have a protection of a risk effect in tubal factor infertility. Low-producing *MBL2* haplotypes are associated with C. trachomatis serology positive tubal factor infertility patients (Laisk *et al.,* 2010). In their study on the role of TLR2 and TLR4 in the development of tubal pathology on knock out (KO) mouse models, Darville et al. (2003) showed that the amount of cytokines produced by macrophages depends on TLR2 but not on TLR4 receptors. Indeed, the deficiency of TLR2 receptors is associated with a decreased production of cytokines in vitro. In vivo, the deficiency or absence of TLR2 causes lower levels of inflammatory mediators, but the course of infection does not differ compared with naïve animals. Microscopic examination of the tubal tissue showed that mice with intact TLR2 are, however, more prone to the development of late inflammatory sequelae. Finally, their study concluded that TLR4 does not modulate innate immune response to Chlamydia, whereas in vivo experiments on TLR2 indicated its important role in protection against late inflammatory sequelae following Chlamydia genital tract infection (Darville *et al.,* 2003). In a study aiming at understanding the role of two TLR2 SNPs in the susceptibility to infection and contribution to the development of the tubal pathology in Dutch women, Karimi et al. (2009) revealed a statistically significant association between certain TLR2 haplotypes and protection from tubal pathology and development of the late inflammatory complications (the absence of TLR2 is associated with an increase in the severity of the Chlamydia infection. As already mentioned, most of the studies assessing host genetic determinants of Chlamydia

infections are focusing on the extracellular TLR2's and TLR4's contribution to the differences in the susceptibility and severity of the infection. However, there is also an interest in the relevance of the intracellular TLR9. So far, human cohort data have not shown significant differences between carriers of mutant alleles and controls in the susceptibility to infection, course of the infection, or frequency of later tubal pathology. On the other hand, experiments in mice models found that TLR9-deficient mice had a higher level of protection against reinfection (Ouburg *et al.,* 2009).

HLA Alleles

In addition to the research directed at TLR genes, there are also indications of association between tubal infertility caused by *Chlamydia trachomatis* and HLA alleles. Cohen et al. (2000 and 2003) found that alleles of the HLA-DQ, DR1, and DRB5 loci modulate the severity of Chlamydial infections. Kinnunen et al. also found that specific HLA-DQ alleles are more frequently present in women with tubal infertility (Kinnunen *et al.,* 2002).
Besides the TLR and HLA alleles, in 2009 Morré et al. published an extensive overview of the then known genetic variants influencing susceptibility and severity of Chlamydial infections including SNPs in cytokines and other pathogen recognition receptors like NODs.

3.2.2. Application of Research

Immunogenetics research on *Chlamydia trachomatis* indicates that a proof of principle for the successful application of genetic and genomic markers for the prediction of late complications after the infection could have a strong public health impact. Subfertility poses an enormous burden on healthcare and society throughout the world. Worldwide, 15% of couples trying to conceive suffer from subfertility (Evers, 2002; Broeze *et al.,* 2010). One of the major causes of female subfertility is tubal pathology (TP) (Evers, 2002), and *C. trachomatis* is the single most common cause for infertility. If left untreated, *C. trachomatis* may lead to ectopic pregnancy, tubal pathology, and ultimately infertility. The cost associated with subfertility is high, as it requires tubal surgery and in vitro fertilisation (IVF). Currently, *C. trachomatis* IgG serology is used to assess the risk of *C. trachomatis*-associated TP in subfertile women (20%) (Figure 1) (Broeze *et al.,* 2010). *C. trachomatis* serology has limited sensitivity and specificity and the predictive value is poor thus, many women undergo additional diagnostic procedures while not needed (40–45%) or do not get intervention while needed (19%). Laparoscopy is widely used to assess the risk of TP in women positive for *C. trachomatis* IgG. This procedure is invasive and expensive (on average 3000 Euros including additional costs) and requires general anaesthesia. Furthermore, it holds a 1.5% risk of surgical complications (e.g., bleeding, infection, or

worse). Therefore it is crucial to develop a companion diagnostic to improve the assessment of risk of TP in *C. trachomatis*-positive and negative women. By doing so, one is able to prevent invasive procedures in patients without TP and reduce both the cost and the psychological burden associated with laparoscopy. This companion diagnostic should merge serology, taking into account serological positivity and titres and considering new serological responses (e.g., pgp3) (Wills *et al.,* 2009) and add the predictive value of host genetic markers involved, for example, related to the innate immune response to pathogens. The genetic trait should consist of a series of markers with a so-called SNP load or gene load linked to decision making for performing laparoscopy or not. Future studies should be directed at performing studies in larger cohorts to access the true clinical potential of this approach.

3.3. HPV

Roughly 20% of cancers are linked to various infectious agents (Roman *et al.,* 2007). Human papilloma virus (HPV) is one of these agents, and the role of different HPV subtypes in the etiology of cervical cancer has been well established (An *et al.,* 2005). HPV infections are in most cases cleared by the actions of the immune system within one year and often remain asymptomatic throughout that period. However, a small percentage of the infections eventually lead to some form of cancer. HPV-induced cancers account for approximately one third of all cancers caused by infectious agents (Lehoux *et al.,* 2009), and HPV is considered to be the most common sexually transmitted infectious agent (Baseman & Koutsky, 2005). However, studies have shown the existence of nonsexual modes of HPV transmission (including transplacental and transmission via fingers and objects (Pao *et al.,* 1992; Tay, 1995; Burchell *et al.,* 2006)), and therefore, HPV cannot be referred strictly to as an STI (Pao *et al.,* 1992).

The HPV virus infects skin or mucosal tissues in the anogenital area or the region of the head and neck. So far more than 100 types have been reported (Lehoux *et al.,* 2009). It has however been proven that approximately 15 out of these 100 types cause virtually all cases of cervical cancer (Syrjanen, 2007). Moreover, HPV types 16 and 18 account for around 70% of cervical cancer cases, and they—particularly type 16—have also been identified in anal, as well as some head and neck cancers (Syrjanen, 2007). The strong association between HPV infection and cervical carcinogenesis makes cervical cancer preventable, thus fulfilling an important criterion for public health relevancy. With the introduction of HPV vaccines, a major breakthrough in prevention has been made. Vaccines proved to be safe and efficacious (Harper, 2009) and vaccination programmes for girls and young women have been implemented in many countries.

3.3.1.Review of the Host Genetic Variants Found to Influence HPV Infection.

Of all the women who are infected with HPV, only a small percentage develops cervical cancer. This observation suggests a role of host genetic factors influencing persistent HPV infections and progression into cervical cancer.

The Role of HLA.

Alleles have been reported to be associated with the development of HPV-related cervical cancer. In their review of evaluating this association, Hildesheim and Wang (2002) found several alleles of HLA class II to be associated with an higher risk of developing cervical cancer (DQB1*03 alleles and DRB1*1501, DQB1*0602). As for HLA genetic variants' protective effect, several studies consistently reported that DRB1*13 and DOB1*0603 are associated with it (Hildesheim & Wang, 2002). Associations between HLA and HPV infection and progression to cancer are reported to be population- and HPV type-dependent. Indeed, HLA DQB1*0301 allele carries an increased risk of cervical cancer in the British population in case of infection with all HPV subtypes (Cuzick *et al.,* 2000), while researchers in Bolivia found a statistically significant association of HLADRB1*1602 with susceptibility to infection (Cervantes *et al.,* 2003).

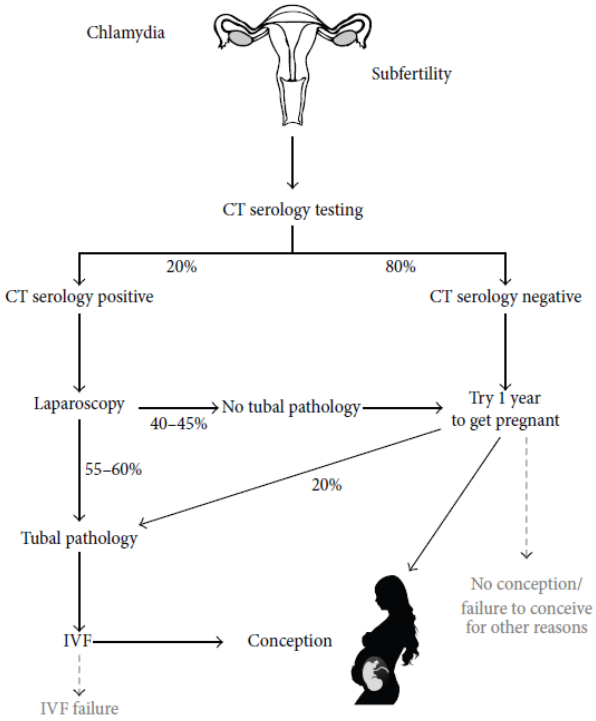

Figure 1. *Current serology protocol for subfertility resulting from C. trachomatis infection. Women with a negative C. trachomatis serology are advised to try to conceive for one year; however, 20% of those women actually have tubal pathology and are thus misdiagnosed. Of the women with a positive C. trachomatis serological test, 40–45% do not have tubal pathology after laparoscopic examination and are thus misdiagnosed. Figure adapted from Lal et al. (2013).*

In their recently published review of the genetic susceptibility to cervical cancer, Chen et al. (2011) presented the most important genetic polymorphisms associated with the development of this disease. Their literature search identified, in addition to HLA genetic variants, genes encoding interleukin-1β, tumor necrosis factor α, interleukin-12 A and B, interferon-γ, interleukin-10, cytotoxic T-Lymphocyte antigen-4, p53, BRCA1, and LAMB3 as those associated with persistent HPV infection and progression to cervical cancer (Chen *et al.,* 2011). In addition, certain genes encoding killer immunoglobulin-like receptors (KIR) also seem to be associated with cervical cancer (Arnheim 2005).

So far, no genetic or genomic applications have been developed based on these findings. When it comes to applying genetic knowledge and discoveries into the field of HPV infection and cervical findings diagnosis and prevention, the strategy known as methylation takes the lead.

3.3.2. The Role of Methylation.

Methylation is a common mechanism through which the silencing of genes, and among these tumour-suppressor genes, can be achieved (Esteller & Herman, 2002). It represents a chemical alteration in regions of DNA referred to as "CpG islands," commonly found in many promoter regions. The alteration leads to the inhibition of the transcription of genes controlled by such methylated promoters (Henken *et al.,* 2007). Methylation markers are easily detected in cervical scrapes, with, for example, methylation-specific PCR (MSP). Hence, positive MSP results in these samples are indicators of methylation of relevant genes in the tissue (Henken *et al.,* 2007). At the moment, the strategy for early detection of cervical neoplasia in screening programmes is cervical scraping cytomorphologic assessment (PAP test), which has a considerably low sensitivity. Data on sensitivity and specificity of the PAP test are highly heterogeneous. Depending on the study done and combination of tests and reference standard thresholds applied, they range from 18% to 98% for sensitivity and from 17% to 99% for specificity (Nanda *et al*., 2012). Furthermore, the National Cancer Institute assessed the sensitivity of the PAP smear to be 55–80% for high-grade lesions and around 68% for low grade lesions (NIH, 2012). Taking this into consideration, there is a need for the development of novel approaches, and additional tools based on methylation markers might be a step forward.

3.3.3. Application of Methylation in Triage of Cervical Carcinomas.

In the study by Henken et al. (2007), 29 tumour-suppressor genes were analyzed as potential methylation targets, and 12 of them were found to have methylated gene promoters in cervical cancer tissue. Eight of those were also associated with consecutive stages in HPV-mediated transformation in vitro. The promoter that was most commonly methylated (in 92% of the examined carcinoma samples) was *MGMT*.

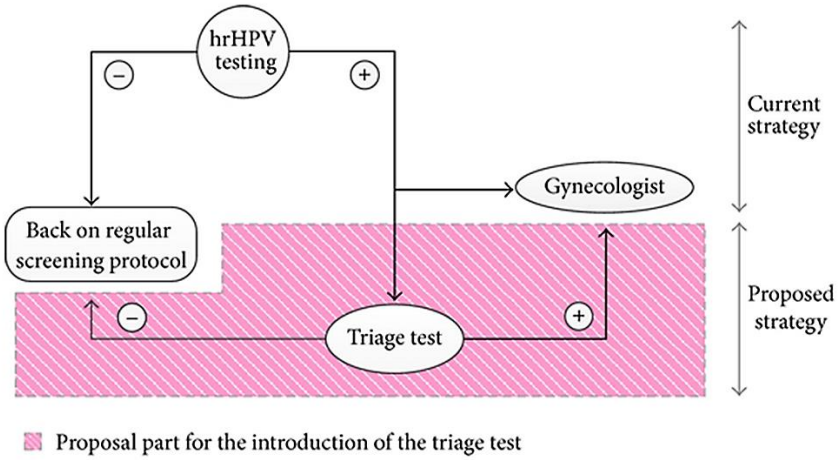

Figure 2. *Introducing methylation as an addition to the primary hrHPV test would lower the number of unnecessary referrals to gynaecologists. Figure based on Yang et al. (2009).*

Methylation of the promoters *CCNA1* and *C13ORF18* in cervical scrapings is found to be strongly associated (< 0.0005) with CIN2 (moderate cervical intraepithelial dysplasia) and higher grade stages of cervical dysplasia, as was determined in the study by Yang et al. (2009). Hence, these would be suitable markers for a triage test, referring a patient to a gynecologist upon a methylation-positive result. The more severe the lesion in the sample, the more methylation was present in these two gene promoters. Analysis of high methylation of these two markers has a high specificity (96% and 100%, resp.), as well as high positive predictive value.

Further, Yang et al. (2009) suggest that their methylation test should be used as a triage test in primary hrHPV testing (high risk HPV test identifies types of HPV which are linked to cervical cancer). hrHPV testing is more effective in preventing invasive cervical cancer; however, it is considered to be less sensitive than cytology in detecting CINs. Introducing methylation as a part of a triage test to the primary hrHPV test would lower the number of unnecessary referrals to gynecologists; especially in younger women who tend to be over diagnosed (Ronco *et al.,* 2010) (see Figure 2).

In another study evaluating the potential value of the methylation markers *CADM1* and *MAL* as a triage tool for hrHPV+ women, it was found that there is a solid reasoning for combining markers that relate to different stages in cervical carcinogenesis (Overmeer *et al.,* 2011). They examined and confirmed the advantage of combining methylation patterns in the promoter region of more than one suppressor gene with the aim to increase the sensitivity for high grade CINs. A methylation-based test focuses on later

phases of the carcinogenesis, given that these promoter alterations increase in these late stages. However, methylation-driven silencing of *MAL* promoter takes place at a very early point, before HPV-positive keratinocytes undergo tumour transformation. Whereas, silencing of *CADM1* promoter by methylation correlates more with late stages. Overmeer et al. demonstrated that this marker combination is optimal for detection of CIN3 lesions (Overmeer *et al.,* 2011). In the process of progression into late stages, there are genes other than oncogenes and tumour suppressors also relevant. MicroRNAs (miRNAs) are short noncoding RNA molecules, which act in regulating expression of protein coding genes, by pairing with sequences within such genes. hsa-miR-124 is an miRNA known to be silenced by methylation in many cancers, and Wilting et al. (2010) proved that this mode of silencing frequently occurs in cervical lesions as well. No methylation was found in normal tissues, while almost 60% was detected in CIN3 lesions, and more than 93%methylation of hsa-miR-124 was present in cervical carcinomas. The methylation of this gene is not directly related to the presence of hrHPV. High positivity is however observed in CIN3 and cervical carcinomas, which altogether makes it a potentially very useful triage marker for hrHPV positive women. This applies however not for setting where HPV genotyping is not implemented yet including under development countries. Triage could serve as an additional step that would more aptly bridge screening and diagnosis in order for a better stratification of women at risk to be achieved (Solomon, 2003). It would be used on those with positive primary screening results to determine the further risk of the progression into later stages. The effects of constructing this type of triage test based on methylation would be expected to land a formidable impact on policies that currently regulate screening intervals.

4. Discussion and conclusion

To our knowledge, this is the first review on the translational potential of basic genomic and genetic findings for HIV, *C. trachomatis*, and HPV into applications in public health and in diagnostics, treatment, and prevention of late complications of these infectious diseases. We found scarce examples of the current application of genomic/genetic findings, in pharmacogenomics, and we found examples of genomic information with a promise of translation in the near future. In our review, we did not focus on analytic validity, clinical validity, and clinical utility and other criteria generally considered to be the most important factors in evaluation of the genetic/genomic applications (Teutsch *et al.,* 2009). Since there are still no market-ready applications, so the aforementioned criteria could not be considered; we focused on an earlier step of this process. We focused on the promising examples of translation of the discovery into a possible application.

Based on the review of the relevant literature some examples can be considered promising. The genes responsible for susceptibility to HIV infection can be basically

divided in two groups, chemokine receptors genes and HLA genes. So far, the discovery of the *CCR5*Δ32 genetic variant opened the door for the development of new anti-HIV drugs. Although undoubtedly a very important step forward, CCR5 targeted therapy and the research behind it are just one of the possible applications of immunogenetic information. Indeed, there is a significant amount of knowledge of certain genetic variants having a positive or negative influence on the course and outcome of HIV infection. Possible future use of the knowledge about the expected course of the infection would be advancing the standard of care and therapy after routine genetic testing.

In the field of *C. trachomatis* caused subfertility there is a promise for a more accurate subfertility diagnosis based on SNPs. Research showed that SNPs in *TLR4* possibly increase the risk of tubal pathology. Specific *TLR2* haplotypes are associated with protection from tubal pathology and development of the late inflammatory complications. These findings, together with the one carrying multiple SNPs in multiple pattern recognition receptors' (PRRs) encoding genes (*TLR9*, *TLR4*, *CD14*, and *CARD15/NOD2*) doubles the risk of tubal pathology in *C. trachomatis* IgG-positive women compared to IgG-positive women carrying less than two SNPs, offer a proof of concept for the development of a genomic application in diagnosis of subfertility. A genetic test as a part of routine subfertility diagnosis should be able to save time and money by decreasing the number of unnecessary laparoscopies and the time patients unsuccessfully spend trying to get pregnant. In the field of HPV, there are some promising advancements in the early diagnosis of cervical cancer based on methylation tests. The methylation markers *CADM1* and *MAL* were found to be an optimal combination for the detection of CIN3 lesions (Overmeer et al. 2011). Moreover, the methylation of *CCNA1* and *C13ORF18* in cervical scrapings is found to be strongly associated with CIN2 and higher grade stages (Yang *et al.,* 2009). A triage test based on such methylation markers might be an important step towards a more effective stratification of patients at risk for cervical cancer. The knowledge about the gene-disease associations should lead to growing numbers of genetic tests, which will in the future have an increasingly important role, in tailored clinical and drug treatment. However, in order for this translation process to succeed, the wide consensus among scientists, clinicians, policy makers, and the industry on necessity of going in this direction needs to be achieved (Burke *et al.,* 2002). Based on what we have shown here, there are many host genetic variants found to have a role in modulating the immune response to HIV, HPV, and Chlamydial infections. However, we found an imbalance between the number of host genetic variants with a role in modulating the immune response and the number of practical genomic applications. Thus, such new knowledge and technologies from basic research are not yet integrated in health in a timely, effective, and efficient manner (Lal *et al.,* 2011).

This imbalance, the lack of translation from bench to bedside, is in favor of basic research that seems to be somewhat hermetic in quality, revealing confirmed positive association

with a certain genetic variant and not exploring the future implications of these findings, should not represent a norm in the field. The next step is needed in which gene-disease association leads to the development of the genetic/genomic application. Starting with interdisciplinary collaboration is very important in the process of evaluation of role of genetic variants in the etiology of human diseases (Little *et al.,* 2002). There are some clear and well-supported genetic associations with particular infectious diseases; these should be driving forces of the successful translation process.

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PART III

SOCIO-CULTURAL FACTORS AND HEALTH LITERACY – NEGLECTED AREAS OF HOST-PATHOGEN RESEARCH, DIAGNOSTICS AND TRANSLATION

CHAPTER 6

Applying a gender lens on human papillomavirus infection: cervical cancer screening, HPV DNA testing, and HPV vaccination

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Abstract

Background

Our aim is to provide a state-of-the-art overview of knowledge on sex (biological) and gender (sociocultural) aspects of Human papillomavirus (HPV) and cervical cancer for educational purposes. Considerable disparities exist in cervical cancer incidences between different subgroups of women. We provide an outline on the crucial issues and debates based on the recent literature published in leading gender medicine journals. Intersectionality was applied in order to help categorise the knowledge.

Methods

Key terms (HPV, cervical cancer) were screened in Gender Medicine, Journal of Women's Health and Women & Health from January 2005-June 2012. Additional searches were conducted for topics insufficiently mentioned, such as HPV vaccination of boys. In total, 71 publications were included (56 original papers, four reviews, six reports, three commentaries, one editorial and one policy statement).

Results

Research reveals complexity in the way various subgroups of women adhere to cervical screening. Less educated women, older women, uninsured women, homeless women, migrant women facing language barriers, women who have sex with women and obese women participate in Pap smears less frequently. A series of barriers can act to impede decisions to vaccinate against HPV.

Conclusions

Both male and female controlled preventive methods and treatment measures should be developed in order to tackle HPV infection and different strategies are needed for different subgroups. A substantial discussion and research on alternative methods of prevention was and is lacking. In future research, sex and gender aspects of HPV-related diseases of boys and men as well as subgroup differences in HPV risk need to be addressed.

Introduction

Sexually Transmitted Infections (STIs) are a formidable public health issue. Many sex and gender differences exist in epidemiology, etiology, diagnosis, treatment and consequences of STIs. There is a clear distinction made between the two – the term 'sex' is reserved for hormonal, chromosomal or any other features stemming from person's biology. Gender, on the other hand, pertains to sociocultural concepts of femininity and masculinity, which can vary between cultures (Verdonk & Klinge, 2012). For instance, besides biological differences related to the reproductive roles of men and women that cause disparities (such as women's higher biological vulnerability for STIs), gender differences occur because women have less power over sexual situations than men (Rosenthal & Levy, 2010). Gender aspects interact with biological sex differences in infectious diseases (Doyal, 2001).

Human papillomavirus (HPV) infection is one of the most common STIs, although it is not solely transmitted sexually, and oncogenic subtypes are associated with cervical cancer, as well as cancers of the head and neck, anal tumours, penile cancers, and cancers of the vulva and vagina (LCI, 2011). Infection with High-Risk (HR) HPV strain is necessary but insufficient for cervical cancer to develop. Currently known co-factors associated with cervical cancer development are cigarette smoking, alcohol consumption, micronutrients deficiency in fruits and vegetables, prolonged use of oral contraception, multiparity, uncircumcised male partner, low socioeconomic status (SES), infection with HIV/AIDS or other STIs including herpes simplex and *Chlamydia trachomatis* (Vetter & Geller, 2007; Thümmler *et al.,* 2009). However, other additional factors may also hinder the immune system in shielding the organism from these infections. Violence compromises the body's ability to counter the infection as well via stress behaviours such as smoking or alcohol consumption (Coker *et al.,* 2009). However, the prevalence of stressful life events or self-reported stress are not always found to be associated with higher risk for cervical disease (Wilkerson *et al.,* 2009). Cervical cancer is also associated with different types of violence: childhood sexual abuse (CSA), intimate partner violence, or forced sexual experiences (Coker *et al.,* 2009). A consequence of CSA is engaging in sexual risky behaviour such as intercourse with casual partners, not using condoms, earlier age of first consensual intercourse, failing to discuss intercourse in advance, having sex with a partner who injects drugs, or having an HIV positive partner (Senn *et al.,* 2006). Studies show that CSA is reported by twice as many women than men (prevalence approximately 30% vs. 15%) and more often by subgroups such as pregnant adolescents, men who have sex with men, lesbian and bisexual women, women in psychiatric care, drug users or persons who tested positive for HIV (Testa *et al.,* 2005; Senn *et al.,* 2006; Austin *et al.,* 2008).

Developments such as the HPV vaccines and the implementation of HPV DNA testing for diagnostic purposes have led to an increase in publications on HPV and heated public

debate. Gender analysis enables us to elucidate different patterns of interaction of gender with race, class, and other factors (CIDA, 2011), which can help us understand how gender relates to HPV infection, HPV vaccination and DNA testing.

Men and women cannot be divided into two homogenous groups, and people's group memberships such as gender, age, ethnic background, sexual orientation, religious affiliation (both assigned and self-identified) redefine each other. Intersectionality is an approach that addresses the way gender and other social identities affect life, and refers to mutually constitutive relationships that influence each other (Crenshaw, 1994; Lanehart, 2009; Hankivsky, 2012). An intersectionality approach is based in social justice, and aims to address processes of inclusion and exclusion in order to address issues of marginalised groups (Hankivsky & Christoffersen, 2008). The approach explains how a single focus on gender, race, or any factor on its own makes it impossible to examine the manner in which social forces and locations interact and relate to each other in order to give shape to the unique mosaic of human experiences. The elements are fluid and flexible, and a subgroup at risk lies at the intersection – the place all relevant elements interact. Gender is therefore essential, but should not be decoupled from other categories, for this would disable the contextual analysis (Hankivsky, 2012). In this literature review we apply intersectionality in order to understand the role gender plays in HPV. Thus, we put a gender lens on HPV and summarise recent publications from three leading journals in gender medicine.

With an eye for intersectionality, we aim to categorise recently published knowledge on cervical cancer screening, HPV DNA testing and HPV vaccination. According to leading journals in gender medicine, what is state-of-the-art in regards to gender knowledge about HPV and cervical cancer?

Materials and methods

The study is funded by the European Union Erasmus Curriculum Development Project Gender Medicine EUGiM (2009–2011). State-of-the-art education material in gender medicine was developed and used during a postgraduate summer school in Gender Medicine, which was piloted in Berlin, Germany in September 2010 and in Sassari, Italy in September 2011.

In order to obtain a good and timely overview in gender and HPV for education purposes, we searched for recent relevant literature in three renowned and leading gender medicine journals: Women & Health, Gender Medicine, and Journal of Women's Health. We opted for the choice of these journals due to the fact that relevant gender-specific publications are often not retrieved by either conventional searches or the use of filters. Moreover, authors who are insufficiently familiar with the field often misuse the terms 'sex' and 'gender'; for instance by applying the term 'gender' even in cases of strictly biological

differences (Oertelt-Prigione *et al.,* 2010). The selected journals, however, cover a broad range of sex and gender-related aspects and tend to the appropriate use of terminology and indexing. Women & Health publishes papers on the physical and psychological health of women, including environmental factors and prevention, historical overviews on women's health and health policy research. Gender Medicine explicitly publishes papers that use sex and/or gender approaches in their design. The Journal of Women's Health takes a multidisciplinary approach to health and illness. Hence, a broad overview of recent research that would include gender aspects of HPV was anticipated. We searched volumes from January 2005-June 2012 and read title and abstracts. This timeframe covers studies on the launch and implementation of the two HPV vaccines and developments in HPV DNA testing. We included papers if the abstracts mentioned HPV, cervical cancer and related issues (risk factors, behaviours, screening and prevention). Additional hand searches were performed in order to provide sufficient information on the HPV vaccine debate and the vaccination of boys. All types of publications were included. After reading the text, we organised the literature based on whether it reports on Pap smears, HPV DNA testing or HPV vaccination. In order to avoid excessive referencing, we reduced the number of cited articles in the text while still focusing on the major factors presented in the literature.

In total, 71 papers were included in this review, out of which 56 original papers, four reviews, six reports, three commentaries, one editorial and one policy statement. The overview of included papers per year is provided in Table 1.

Publication year	*Cervical cancer screening*	*HPV DNA testing*	*HPV vaccination*
2005	**2** (Fish et al.; Grinstead et al.)	**3** (Anhang et al.; Cooper et al.; McCree & Dempsey)	**0**
2006	**3** (Eggleston et al.; Lindau et al.; Manderson & Hoban)	**4** (Bradley et al.; Perrin et al.; Philips et al.; Sharpe et al.)	**2** (Zimmerman; Calloway et al.)
2007	**2** (Bolen et al.; Nash et al.)	**1** (Savard)	**7** (Coker et al.; Godfrey et al.; Lippman et al.; Markman et al.; Rambout et al.; Rosenthal et sl.; Vetter et al.)
2008	**5** (Eaton et al.; Peterson et al.; Schnatz et al.; Schutt et al.; Seaver et al.)	**0**	**1** (Casper et al.)
2009	**12** (Althoff et al.; Bharel et al.; Brewer et al.; Clark et al.; Corliss et al.; Coker et al; El-Hammasi et al.; Fisher & Brundage; Kavoussi et al.;Nelson et al.; Steele et al.; Zhang et al.)	**0**	**9** (Akinsanya-Beysolow et al.; Carpenter & Casper; Casper & Carpenter; Conroy et al.; Foresta *et al.,* Hull & Caplan; Leader et al.; Pitts et al.; Sanderson et al.)
2010	**6** (Guilcher et al.; Lee et al.; Leung et al.; Nijhawan et al.; Tracy et al.; Wang et al.)	**0**	**3** (Askelson et al.; Dillner et al.; Wong et al.)
2011	**2** (Ramaswamy et al.; Smith et al.)	**0**	**4** (Gerend & Sheperd; Hutson et al.; Kang & Kim; Mills et al.)
2012	**1** (Montgomery et al.)	**1** (Rijkaart et al.)	**4** (Jim et al.; Naleway et al.; Saraiya et al. Tiro et al.)
total	**33**	**8**	**30**

Table 1. *Numbers of included papers per each year with publications specified in brackets*

Results

The following sections present main findings from the published research on cervical cancer screening, HPV DNA testing and HPV vaccines, with an additional focus on the debate over HPV vaccination and the issue of vaccinating the boys.

Cervical cancer screening

Differences in HPV prevalence and cervical cancer screening rates between subgroups of women may exist due to a mix of patient characteristics, healthcare factors, and patient and physician attitudes. From 1995–2005 in Europe, cervical cancer incidence and mortality has declined, although in some countries it increases, such as Lithuania, Romania, and Bulgaria (Thümmler *et al.,* 2009). Globally, the Pap test is the principal means of detecting abnormalities, although organisation of screening programmes, coverage, eligibility criteria and recommended screening intervals differ. The Pap smear

has become routine and reduced the incidence and mortality of cervical cancer (Fisher & Brundage, 2009), but cervical cancer still causes significant morbidity and mortality in developing countries. For instance in Nigeria, the prevalence of HR HPV and cytological abnormalities in women is 16.6% and consistent with other regions in Africa (Schnatz *et al.,* 2008). In American studies, Latinas were less likely to die of the disease than other groups (Eggleston *et al.,* 2006). Black women diagnosed with localised cervical cancer less likely had surgery, suggesting less optimal treatment (Coker *et al.,* 2009). In New Zealand, ethnic disparities in cancer survival are reported as well: Maori women were diagnosed more often with late stage cervical cancer, and had shorter survival, although excess mortality decreased over the years 1994–2005 (Brewer *et al.,* 2009). Both SES and ethnic background are associated with poorer survival.

In many countries, screening programmes are set up for adult women at certain intervals. Overall, women who maintain on-schedule Pap tests appear to the generally healthier than women who do not obtain regular Pap smears (Nelson *et al.,* 2009). In the US, women are advised to undergo annual screening or every 3 years if they had three consecutive normal Pap tests (Cooper *et al.,* 2005). Free cancer screening programmes exist for low-income and uninsured women (Peterson *et al.,* 2008). Different factors and their mutual interplay appear to play a role in cervical cancer screening rates. The overview of studies in which a number of relevant factors were observed, together with the explanation of the manner in which the factors affect the screening rates, is given in Table 2.

Factors	*Major observations*
Knowledge	– a highly consistent factor contributing to higher participation of women in Pap screening (Peterson *et al.,* 2008; El-Hammasi *et al.,* 2009; Leung & Leung, 2010)
SES	– low socio-economic status is associated with higher cervical cancer rates, lower Pap smear rates, and inadequate follow up (Peterson *et al.,* 2008; El-Hammasi *et al.,* 2009; Bharel *et al.,* 2009) – women age 50+ with higher education are increasingly more up-to-date regarding screening services with each educational level (Bolen *et al.,* 2007)
Healthcare, access to healthcare, insurance	– not having health insurance is associated with not having a recent Pap test in southern US women (Peterson *et al.,* 2008) – universal healthcare appears to contribute to the reduction of socio-economic status related differences or differences in screening based on residential location (Smith *et al.,* 2011) – an older study however showed that social factors discourage Australia's Indigenous women's use of and access to health services for screening, diagnosis and treatment of cervical cancer (Manderson *et al.,* 2006)
Age	– younger women age 19-26 exhibit more knowledge and participate more in preventive practices than women age 40-70 (Montgomery & Smith-Glasgow, 2012)
Marital status	– participation is higher in married women in Kuwait compared to unmarried women (El-Hammasi *et al.,* 2009)
History of cervical infection, family history	– higher prevalence of ever having a Pap test is observed in women with either personal or family history of cancer (El-Hammasi *et al.,* 2009)
Health expert's willingness to give screening recommendation	– physician's recommendation is one of the strongest predictors of having had a Pap test (Peterson *et al.,* 2008)
Lifestyle	– smokers and obese persons adhere to Pap testing less frequently (Nelson *et al.,* 2009)

Table 2. *Various factors influencing the participation rates of women in cervical cancer screening*

With regards to HPV prevention, screening and treatment, many subgroups of women are underserved such as women living in rural areas, lesbian women, older women, or women with low health literacy levels. Cancer screening programme satisfaction is a critical outcome for the healthcare system, and for patients with ethnic background, SES and health status play a role in patient satisfaction (Schutt *et al.,* 2008). They often have to be addressed in a language not sufficiently familiar to them.

The findings on higher frequency of testing in African American women in the south of the US (Peterson *et al.,* 2008) may be an indicator that the racial gap in receiving Pap smears in the US is closing. The fact that non-Hispanic whites adhere less to Pap screening might be an outcome of years long campaigning within minority communities (Nash *et al.,* 2007). To date, experiences of maltreatment were informative of black people's skepticism towards healthcare, such as side effects of HPV vaccine (Scarinci *et al.,* 2007). Within the Asian American community there are prominent differences, with the Korean subgroup showing the lowest screening rates. Japanese American women participate in cervical cancer screenings considerably less compared to other cancer screenings (Lee *et al.,* 2010). Chinese women in the US have lower screening rates mainly due to language barriers and lack of general knowledge, however education and interactions with a

Chinese physician largely increased screening rates (Wang *et al.,* 2010) . In China, women's status is inferior to men's, and women are more likely to live in poverty. SES affects Chinese women's health care seeking behaviour for self-reported genitourinary symptoms. Contrary to conventional wisdom and possibly due to more knowledge and awareness of stigma, Chinese women with high SES may be just as likely or even less likely to seek treatment for their symptoms (Zhang *et al.,* 2009).

Women who have sex with women (WSW) or lesbian women have lower screening rates even though HPV is transmittable between female sex partners and WSW often do also have (occasionally) sex with male partners (Eaton *et al.,* 2008; Clark *et al.,* 2009). WSW's risk perceptions for HPV are lower than expected given the prevalence of abnormal Pap smears and HPV diagnosis, and thus, characterised by optimistic bias (Eaton *et al.,* 2008). WSW may not seek out routine care by physicians, partly because they do not need prescriptions for contraception. Experiencing the healthcare system as being heterosexist evolves from the assumed heterosexuality evident in waiting rooms, health forms, and healthcare providers' assumptions (Seaver *et al.,* 2008). Fear of discrimination plays a role in delaying healthcare seeking (Tracy *et al.,* 2010). In Canada, lesbian and bisexual women reported poorer health status on several health measures, but bisexual women negatively stood out. This group particularly appears to be not well served in Canadian healthcare [54], which was also reported in the US (Corliss *et al.,* 2009). In the UK, lesbian women are more likely to avoid screening than heterosexual women, and more likely to have never attended screening than American lesbian women (Fish & Anthony, 2005). In the US, regular cervical cancer screening rates were equal among women partnering with men, women, or both (Clark *et al.,* 2009). Although a high percentage of lesbian women engage in sex with women and men, lesbian women may have a lower cervical cancer risk. However, lesbian women are more frequently obese and smoke more often, which may increase their cervical cancer risk (Tracy *et al.,* 2010). Other subgroups of women at risk are presented in the following Box.

Perimenopausal women:
- increased HPV prevalence (Althoff *et al.,* 2009)
- both exogenous and endogenous hormones were associated with HPV infection (Althoff *et al.,* 2009)

Women over 55 years of age:
- rarely initiate conversations on sexual matters (Lindau *et al.,* 2006)
- physicians tend to initiate these discussions more with African American women (Lindau *et al.,* 2006)

Incarcerated women:
- these women (but also incarcerated men), as well as those with a partner being released from prison are at risk due to their sexual behaviour (Grinstead *et al.,* 2005)
- more frequent history of abnormal Pap smears, particularly those exposed to violence (Ramaswamy *et al.,* 2011), however they are also more receptive to prevention (Nijhawan *et al.,* 2010)

Women with disabilities:
- may have lower screening rates due to difficulties associated with pelvic examinations (Guilcher *et al.,* 2010)
- Canadian women with traumatic spinal cord injury did not have lower screening rates, possibly due to frequent visits to physicians (Guilcher *et al.,* 2010)
- liquid cytology Pap smears are a reasonable alternative for screening this subgroup of women (Kavoussi *et al.,* 2009)

***Box**. Different subgroups of women at heightened risk from cervical cancer*

HPV DNA testing

Current infections can be measured with highest sensitivity by HPV DNA testing, which can also be combined with Pap smears for optimising detection of high-grade cervical intraepithelial neoplasia (Rijkaart *et al.,* 2012). Women with a negative HR HPV DNA test and a negative Pap test can extend their Pap screening interval (Cooper *et al.,* 2005; Sharpe *et al.,* 2006; Savard, 2007). Protocol redesign could be focused on those at high risk and be more cost-effective than existing protocols (Philips *et al.,* 2006). However, a few questions still remain. First, a Pap-plus-HPV test has higher sensitivity, but lower specificity, and thus raises the false-positive rate together with the true-positive rate which may result in over-treatment. Secondly, the high prevalence of transient infections among young women is high. And thirdly, a higher number of women with persistent infections but normal cervices will be detected. Therefore, a Pap-plus-HPV test may be more appropriate for women with borderline or mild Pap abnormalities, because a negative HPV DNA test could reassure women that their Pap test result is likely to be aberrant whereas treatment for women with a positive HPV DNA test may be started more rapidly (Philips *et al.,* 2006). In the United Kingdom, before introduction, HPV testing was perceived as an added value and would not negatively affect participation in Pap screening (McCree & Dempsey, 2005). Informing women of their HPV status has both benefits and risks. A positive HPV test result may promote safer sexual practices; empower to adhere to Pap screening and follow-up; or engage in actions to prevent genital warts or cervical cancer. On the other hand, negative psychological side effects can occur such as stigma, blame and shame (McCree & Dempsey, 2005; Perrin *et al.,* 2006). Self-collecting vaginal samples to test for HPV DNA seems feasible and counteracts

barriers to screening attendance such as uncomfortable or anxiety-provoking pelvic examinations and contact with providers (Anhang *et al.,* 2005). Self-collection of an HPV DNA test was highly acceptable among women because it was easy to perform, not painful, private, and could be conducted by the women themselves. However, the highest acceptability rates were found in subgroups with the highest screening rates already. More educational efforts are needed as many women were concerned whether they had done the self-collection test correctly (Anhang *et al.,* 2005).
In developing countries, where screening is often ineffective or under-utilised if existent at all, screening by female nurses appeared to be highly acceptable (Bradley *et al.,* 2006). New screening methods such as HPV DNA testing, but also new visual inspection methods, provide opportunities for low-resource settings, decrease the number of visits of patients, and can be managed by mid-level healthcare providers (Bradley *et al.,* 2006). New genetic technologies may increase our knowledge not only on pathogen genetics but also on host genetic factors as regards HPV. Individual genetic susceptibilities may exist with regards to HPV infection and progress into cervical cancer.

HPV vaccine

A Pap smear detects abnormalities after they occur, thus pre-cancerous lesions may be missed. Therefore the discovery of the link between HPV and cervical cancer, and hence, the possibility of immunisation, enabled primary prevention. In sharp contrast with routinising Pap smears, the medical community, policymakers, and the public showed rapid attention to the HPV vaccines (Fisher & Brundage, 2009). In 2006, the US Food and Drug Administration (FDA) approved of a noninfectious recombinant quadrivalent HPV vaccine (Gardasil) targeting HPV-16 and 18 as well as HPV-6 and 11 against genital warts for use in girls and women aged 9–26. In 2008, the second vaccine (Cervarix) targeting HPV-16 and 18 was marketed. In the US, the Center for Disease Control (CDC) and the American Medical Women's Association recommend that women are vaccinated against HPV (Zimmerman, 2006). Young women are targeted for two reasons. First, immunological response is strongest in girls aged 10–15, and secondly, the vaccine is most efficacious in women who have not yet had sex (Vetter & Geller, 2007). The acceptability of the vaccine is decisive to its implementation (Zimmerman, 2006) and insurance coverage predicts HPV vaccination, for instance among young, low-income, urban, predominantly black women. Also, abnormal Pap test in the past and normative beliefs that medical providers, parents, and others approve predicts HPV vaccination as well (Conroy *et al.,* 2009). A study of young rural women revealed however that those with a reported history of an abnormal Pap test or of never having had a Pap test declined free HPV vaccination more frequently and vice versa. Higher refusal was also observed in

women engaging in behaviours that increase the risk of HPV infection, e.g. mutual masturbation (Mills *et al.,* 2011).

Low awareness of HPV exists among women and men across different age categories, geographical locations and racial/ethnic backgrounds (Vetter & Geller, 2007; Pitts *et al.,* 2009; Kang *et al.,* 2011). Even in groups that demonstrate very high HPV awareness, such as young women at universities, the knowledge is often moderate. This particularly pertains to Latinas and women opposed to premarital sex (Gerend & Sheperd, 2011). In a study of the US girls aged 11–26 receiving their first injection, accurate knowledge on HPV and HPV vaccine increased with their age (Naleway *et al.,* 2012). Hutson and colleagues (2011) explain how the HPV knowledge gap is present across a broad age spectrum – in women aged 18–49. In their study of Appalachian women they describe how the absence of knowledge acts as a strong barrier to vaccination. The study revealed their greater concerns over vaccine's side effects rather than over potential promiscuity effect (Hutson *et al.,* 2011). Latina mothers' acceptability of the HPV vaccine for their children was generally high, even higher among HPV-positive mothers (Sanderson *et al.,* 2009). In Singapore however, women had low levels of awareness and incomplete knowledge of HPV, but acceptability of HPV vaccination was high, although acceptability to vaccinate their children was lower for HPV vaccination than for other preventable diseases (Pitts *et al.,* 2009).

In a survey study among family practitioners, general practitioners (GPs), and pediatricians, their agreement with professional recommendations was the most important variable determining intention to vaccinate young female patients (Askelson *et al.,* 2010). Gynecologists and obstetricians would not offer vaccines mainly because of the costs and the belief that others should provide vaccines (Akinsanya-Beysolow *et al.,* 2009). A recent CDC report on the US physicians' knowledge on the types of cancer that HPV vaccine is effective against (Saraiya *et al.,* 2009) revealed their lower awareness that the vaccine presents not only cervical, but also vaginal, vulvar and anal cancer. Only around one quarter of respondents were aware of the effectiveness of the vaccine in preventing these other types of cancer. Health service providers working with American Indian/Alaska Native populations (who have a higher rate of cervical cancer incidence compared to non-Hispanic white women) often think that a pregnancy test should precede HPV vaccination. Also, they are more reluctant to vaccinate younger patients, which is of concern given the optimal age when the vaccine exerts maximum benefits (Jim *et al.,* 2012).

Other barriers, such as costs to parents and difficulties getting adolescents to primary healthcare providers, are still unsolved in many countries. Lower vaccine initiation was associated with having parents with low incomes, having public insurance, and having fewer sexual partners (Tiro *et al.,* 2012).

Debating HPV vaccines

Despite the enthusiastic reception of the HPV vaccines by women's health advocates, women at risk, and public health officials, an intense debate has taken place (Markman, 2007). A major concern raised is the vaccines' effect on screening practices. Clinicians need to understand the difference between HPV vaccination to prevent cervical dysplasia and vaccinations for childhood infectious diseases, as effectiveness must be evaluated over decades (Godfrey, 2007). Anti-vaccinationists questioned the vaccines' safety and efficacy (Casper & Carpenter, 2008), and the assumption that there is one age at which all girls are negative for HPV fails to account for the girls who acquired HPV not through sexual contact, or for the 10-15% of sexually abused girls (Godfrey, 2007). US conservatives framed the vaccine as 'the promiscuity vaccine' and feared that vaccinating preteen girls would sabotage their message of abstinence from sex before marriage by 'disinhibition effects', although such claims were countered by the CDC. Nevertheless, the debate has influenced public opinion about HPV vaccination and the percentage of parents in favour of mandatory HPV vaccination has declined. The relationship of the HPV vaccine with the evocation of a young/teenage women's sexuality has had a particular effect which was not seen in other vaccines, for instance Hepatitis B (Casper & Carpenter, 2008; Carpenter & Casper, 2009).

Others claim that vaccination violates parents' rights (Vetter & Geller, 2007; Casper & Carpenter, 2008). Intentions to vaccinate seem highest when the vaccine is presented as of little or no cost to women's family and as preventing cancer, and when HPV was not described as an STI. Costs are thus a realistic barrier for intention to vaccinate and stigma still follows HPV (Leader *et al.,* 2009). In Australia, high levels of acceptance exist towards vaccination but reservations emerged when women understood the association between HPV and sexual activity (Rosenthal *et al.,* 2007). In other countries, the debate is more to be framed as anti-vaccinationist and questions were raised about the vaccine's long-term protection or validity in the 'real world'. A systematic review on HPV vaccination shows that it is highly efficacious in preventing HPV infection and precancerous cervical disease, but long-term follow up to substantiate reductions in cancer incidence and mortality is needed in more representative populations of women (Rambout *et al.,* 2007). Two RCTs to evaluate the effect of the quadrivalent vaccine in preventing cancerous lesions and genital warts showed sustained protection against low grade (I) lesions attributable to one of the four HPV types - 6, 11, 16 and 18 - and a substantial reduction of the disease burden 42 months follow-up (Dillner *et al.,* 2010). The most frequently reported adverse effects are pain at the injection site (78%), bruising or discoloration (17%), swelling (14%), as well as fainting (15%). These reactions were reported more in younger girls than the older ones [83]. The vaccine is safe and well-tolerated, although side effects can only be ruled out at large numbers of vaccines (Vetter & Geller, 2007). A study using the Vaccine Adverse

Event Reporting System to identify cervical cancer and carcinoma in situ among vaccinated women showed a few cases of cervical dysplasia and carcinoma in situ (Wong *et al.,* 2010). The women may have been exposed to HPV before vaccination, or to other HR strains. Moreover, vaccine failures may occur (Wong *et al.,* 2010).
In Canada, other argumentations were heard. Women's health advocates said there were too many unanswered questions and since cervical cancer in Canada has been declining, no cervical cancer epidemic exists to warrant the urgency of a vaccination programme. They warn not to overstate the relationship between HPV and cervical cancer because most HPV infections clear spontaneously (Lippman *et al.,* 2007). Only 2-5% of all women with a cancerous HPV infection will develop cervix carcinoma within 12–15 years (LCI, 2011).

Boys and HPV vaccination

Little attention is paid to whether boys should be vaccinated against HPV as well (Hull & Caplan, 2009). Boys are not targeted for vaccination, which is hardly debated. For instance, in half of the US newspaper articles on the HPV vaccine men were not mentioned as possible recipients (Calloway *et al.,* 2005). But immunity of the population, or herd immunity, can only be reached with a gender-inclusive vaccination policy given the fact that men play an important role as STI carriers (Hull & Caplan, 2009). Another question is why there is so little knowledge available on male specific HPV-related disease to begin with. HPV seems associated with testicular cancer as well as with reduced sperm motility and infertility in men (Foresta *et al.,* 2009). HPV-related disease such as oropharyngeal cancer, conjunctival squamous cancer, genital warts and anal and penile cancer does occur among some groups of men, such as men who have sex with men (MSM) and HIV positive men (Godfrey, 2007; Foresta *et al.,* 2009). Proposals that boys should receive HPV vaccine to prevent cancer in women are far more common than claims about preventing penile and anal cancer in men (Foresta *et al.,* 2009).

Discussion

In addition to biological sex differences (Doyal, 2001), sociocultural gender differences, as well as their interaction at many levels, predict HPV risk and risk behaviour, healthcare access and screening rates, and consequences. In biomedical sex and sociocultural gender research, an intersectional approach is the next step forward (Verdonk & Klinge, 2012); it provides knowledge on how men and women's different positions on aspects such as SES, ethnic background, or sexual orientation are interdependent and influence STI risk and health. According to Hankivsky et al. (2010), intersectionality directs attention to health conditions that are more serious for certain groups of women and men. The relevance of

sex and gender cannot be assumed a priori – other crucial aspects, such as SES, might be overshadowed by doing so (Epstein, 2007). Using pre-selected categories (for instance men and women) and treating them as homogenous and detached from other variables would be counterproductive, as they redefine each other (Hankivsky, 2012). Gaining insight into what happens within and between different groups takes research one step beyond subgroup analyses, which assumes categories such as gender or SES as independent categories. However, unequal social relations are incorporated in broad systems of historical inequalities which intersect, overlap, and reinforce each other to shape a person's health status. Their connections to institutions such as healthcare, education, and law can be the target of intervention rather than individuals, in order to improve health (EC, 2012). We aimed to provide a comprehensive compendium of HPV-related health issues, diagnostics and prevention and present this knowledge by applying this approach. To our knowledge, there are no studies applying gender analysis coupled with intersectionality approach to this topic. By targeting the complexity of the intersections we aimed to shed light on particular subgroups which should be targeted when dealing with health disparities.

Our study points out several main findings. It shows that little knowledge of particularly men's health in HPV seems available in gender medicine journals, despite the obvious relationship between gender and STIs and despite the definition of gender as a relational concept. Existing knowledge about men seems to focus on MSM, which has the potential to stigmatise MSM, and leaves men who have sex with women as well as those who have sex with men and women out of the picture. However, in every health condition and even more so in STIs, men and women's health are intertwined. Less educated women, older women, uninsured women, homeless women, migrant women facing language barriers, WSW and obese women in most societies participate less often in Pap smears. Certain groups of women who were traditionally at a higher risk of cervical cancer (e.g. African American women) appear to be benefiting from community-based awareness-raising programmes, as a drop in prevalence can be observed. Other groups at increased risk, like homeless women, decline free Pap smears nevertheless. Thus, reasons for lower screening rates among some (sub)groups are evidently complex. Also, within those groups known to be at higher risk, for instance older women, we can observe the positive effect of education on screening participation. This bares implication for policy development and improvements, by helping to narrow down the focus of intervention to those less educated, therefore using the resources more effectively.

Our method yielded many papers on HPV and HPV related topics, mainly due to the launch of the HPV vaccine in this time period. Studies on awareness and attitudes in different (sub)groups are still frequent in journals with a gender focus. Since we chose to incorporate papers from three journals, we may have missed certain aspects in our overview. The men's health movement advocates research in men's health issues and in

the past years several journals have been established in this field. Future research may focus on their scope in the field of STIs and HPV.

The European Commission requires attention for gender aspects in health research, and EU-funded researchers must address sex and gender research in their designs (EC, 2012). This study is but one example of why that ought to be an imperative. Our overview does provide input for a research agenda for the benefit of both men and women's health. First, research on boys and men pertaining to HPV, both biomedical and gender research, needs to be accelerated. Furthermore, subgroups of men need attention, such as men with many sex partners, ageing men who use sildenafil, and also men who have sex with men and women. Lastly, research should also aim for a better understanding of how synergies of social factors contribute to both men and women's HPV risk.

Conclusions

As regards HPV prevention, screening, and treatment, many subgroups of women are underserved such as women living in rural areas, WSW, older women, or women with low health literacy levels. Often those at higher risk of cervical cancer tend to show lower awareness and knowledge regarding the HPV virus and HPV vaccine. In acquisition and transmission of HPV, gender relations are essential because of: physical and biological aspects; structural factors such as access to healthcare; social differences between men and women and unique interplays of social factors for each subgroup. Research on the role of host genetic susceptibilities may yield more insights on the risk factors in the near future. Various measures for preventing acquisition and transmission of STIs, such as male circumcision, need to be more seriously considered and with an open mind (Rennie *et al.,* 2007). Female controlled methods (for instance female condoms, microbicides, and diaphragm) can be initiated by women themselves, which could make them a powerful resource for women to protect their own health (Thorburn *et al.,* 2006; CHANGE, 2010). However, these measures to reduce transmission of STIs are still studied less often and are still undergoing clinical studies (CHANGE, 2010). In order to target STIs and HPV infection, both male and female controlled preventive and treatment measures need to be developed and different strategies are needed to reach subgroups of men and women. An open mind towards different strategies for prevention of HPV and a conceptualisation of gender as a relational and intersectional concept in which the health of men and women impact each other is of major importance.

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CHAPTER 7

Genome-based health literacy, a new challenge for Public Health Genomics: translation of host genetics into subfertility diagnostics

Adapted from:

Genome-based health literacy – a new challenge for Public Health Genomics

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Abstract

So far, health literacy has not been sufficiently discussed in the context of Public Health Genomics. Primarily not genomic, but genome-based health information needs to be addressed taking into account genome-environment interactions and integrating all health determinants including genomics into a systemic and holistic approach. Translating findings from epigenomics and systems biomedicine will help to understand that individual biological pathways or networks are permanently interacting with environmental networks such as social networks. By this, in the end also health literacy will become personalised. Here, we focus in more depth on the use of host genomic knowledge and how it may personalise clinical management of patients, through the example of subfertility diagnostics. Such implementation would put specific demands on health specialists as well as patients. Genome-based health literacy is challenged by the question which information is relevant for an individual/patient or a health professional, for what purpose and in what context. Public health tools and expertise already in place can and should be used to tackle these huge challenges.

Introduction

Lately the healthcare systems are facing considerable changes due to the rapid advances in different branches of basic sciences including genomics, especially epigenomics, and systems biology. It is obvious that Public Health Genomics needs to translate these advances in a responsible manner and effective way into public health as the new understanding of the causation and pathways of diseases enables healthcare systems to modify their prevention strategies (Brand *et al.,* 2007). We are now moving from the era of genetic testing and genetic screening of primarily Mendelian disorders towards a more holistic and integrative approach that considers genome-environment interactions as well as a multitude of gene variants and their association with diseases formerly thought to be very different and unconnected. This shift in approaches is driving healthcare towards personalised format of patient management. Also, the field of host genomics is making significant progress in elucidating the role of networks of genes regulating immune responses to infections and related diseases and complications (Malogajski *et al.,* 2013). Successful implementation of these factors for the purpose of genetic testing carries the potential to change diagnostics and therapeutics of these diseases.

These innovations are going to affect not only medical services, but also public health as a whole. Genome-based information and related technologies will provide a possibility to identify individuals or subgroups at risk for developing health problems in a very early stage. Thus, the prevention and treatment strategies used in the current public health systems will be challenged and need to be communicated in a proper and timely manner not only to the health professionals, but also to the general public, health policy-makers and other stakeholders involved in the various tasks of public health.

Health literacy is a relatively new, but increasingly important task of public health around the world. The main value of the field is in its multidisciplinary character. Uniting health and medical professionals with the educational specialists creates the possibility to communicate health information to the general public by accessing, understanding, appraising and applying all forms of information for sound health decision-making. This article aims to describe the possible implications genome-based health information might have for the different stakeholders' health literacy. Already now genetic counselling, which is traditionally used in the setting of clinical genetics, aims to increase genetic health literacy not only in affected persons and families, but also in the general public, and is considered to be important as an educational strategy, as well as a way to provide support and reduce psychological distress (Evers-Kiebooms & Vanden Berghe, 1979; Veach *et al.,* 1999; Meiser & Halliday, 2002; Braithwaite *et al.,* 2004; Wang *et al.,* 2004). Although some aspects and experience are highly valuable, genetic counselling as such is too narrow to be used in the context of public health and Public Health Genomics. Thus, in the following, special attention will be paid to the changing role of the

patient/customer/individual and the specialist. Also, the role of understanding genome-based information and its implications in health care by both the patient and the specialist will be covered.
We will start by defining health literacy and in particular genome-based health literacy. Then the relation between public health systems and the place of health literacy within public health will be discussed using the approach of the public health wheel. We will outline the most critical points to take into consideration in regards to the implementation of genome-based technologies into the clinical setting. The potential challenges that patients might encounter upon successful implementation of genome-based technologies will be discussed, as well as their specific health literacy demands in respect to these technologies. Finally, conclusions will be drawn and the direction for the future research and education will be mapped.

From health literacy to genome-based health literacy

Health literacy is the ability to understand health information and to use that information to make good decisions about one's health and medical care (Bowling *et al.,* 2008; MedlinePlus, 2010) defined genetic literacy as "sufficient knowledge and appreciation of genomics principles to allow informed decision-making for personal well-being and effective participation in social decisions on genetic issues." This definition is similar to others proposed in the literature (McInerney, 2002).
According to the Institute of Medicine, health literacy has the following components: oral literacy (listening and speaking skills), print literacy (reading and writing skills), and numeracy (basic quantitative skills), in addition to cultural and conceptual knowledge (Nielsen-Bohlman *et al.,* 2004).
In Europe the public health consortium of the European Health Literacy Project (HLS-EU) defines four dimensions of health literacy: Accessing (1), Understanding (2), Appraising (3) and Applying (4) information in all forms to make health decisions in everyday life throughout the life span (HLS-EU, 2009). These four dimensions demonstrate that health literacy is not "one uniform approach", but has different specific aspects, which can be systematically tackled at different levels within in public health.

Public health as the context for health literacy

As it was described above, health literacy is a vitally important component of every public health system. However, nowadays the main target of health literacy is the general public. Shortly after empirical studies showed, that when consumers are meaningfully engaged in the process of making decisions about their own medical care, the health outcomes are measurably improving (Greenfield *et al.,* 1988; Brody *et al.,* 1989; Kaplan *et al.,* 1989), the

new trend has picked up in public health. Previously the healthcare providers believed that it is their responsibility to make decisions about their patients' health for their benefit, as they are the ones possessing the knowledge about it (Hiller *et al.,* 1997). Currently more and more attention is paid to educating the lay people how to make meaningful choices about their health.

It is important to note that educating general public is only one of ten tasks of public health. The whole variety of the public health tasks is best described and widely used through the public health wheel, which is based on the findings in the report "The Future of Public Health" prepared by the Institute of Medicine (IoM, 1988). In this report the three core functions of public health were defined: assessment, policy development and assurance. Later these three areas were specified into the so-called "ten essential public health tasks" (Public Health in America, 2008).

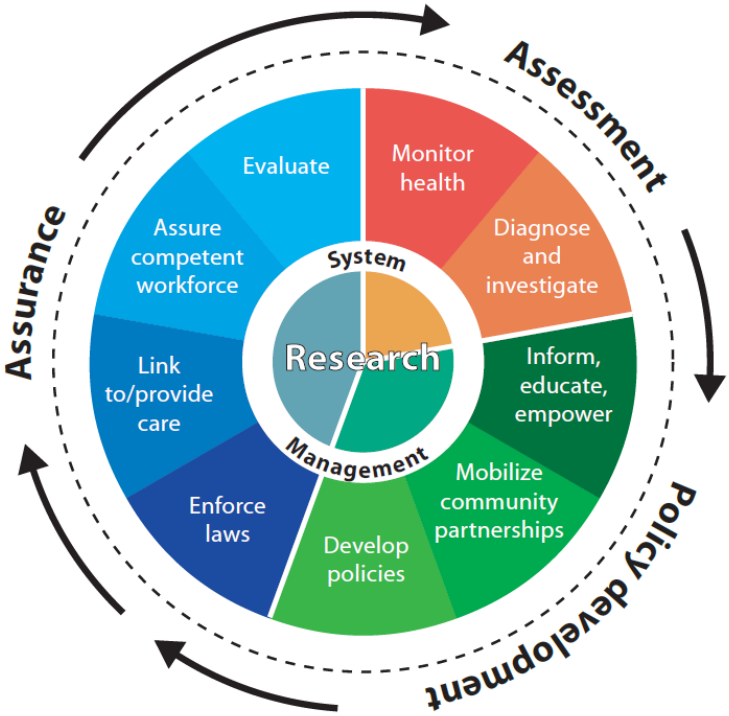

Figure 1. *the public health wheel with the ten essential public health tasks (IoM, 1988)*

Looking at the public health wheel, it is quite obvious, that health literacy is not "just" education. It can be seen as one of the on-going key tasks of public health covering several of the ten essential public health tasks of the wheel including informing, educating and empowering people.

As a consequence, also health literacy about genome-based innovations covers a wide range of topics: genome-based health literacy includes knowing not only about benefits, risks, and limitations of the traditional genetic screening and testing, but also about the implications of genome-based health information as a whole. This implies knowledge about the terminology and technologies of modern genomics, the social and psychological implications of modern genomics for the individual using this information as well as for family members.

Thus, following the public health wheel, a variety of genome-based health literacy actions can be taken such as:

1. *Inform, educate and empower*

This task can include the education of the general public regarding genome-based health interventions through campaigns or mass media, in class education of the different groups, public lectures for the representatives of the public, personalised advice etc.

2. *Mobilise community partnerships*

Here health literacy of both the public and the representatives of the authorities on the local or regional level is important in order to reinforce the cooperation.

3. *Develop policies*

At this stage attention should be paid to health literacy of the relevant public policy officials involved in Public Health Genomics in order to narrow the gap between the dynamics of basic science and the policy-making. It can also include bringing together for a consultation process the public with representatives of other stakeholders.

4. *Link to\provide care*

It is not only the public who are indirectly connected with healthcare and who need to be capable in interpreting genome-based health information. *Health literacy of the health professionals on all levels of the healthcare system is of crucial importance for the translational process.* Studies show that the knowledge of general practitioners, specialists and nurses about genomic advances and their added value for health interventions is very limited (Baars *et al.,* 2005a; Teng & Spigelman, 2014). Moreover, a Dutch study demonstrated a considerable lack of genetic knowledge in medical students nearing graduation as well (Baars *et al.,* 2005b). In that respect, in order to achieve effective introduction of genome-based knowledge and technologies health literacy should start from the very basic level of healthcare provision.

Genome-based health literacy: some challenges are quite unique

As genomic research is now also focusing on multifactorial diseases, new issues in communicating with patients and the general public about genetic contributions to disease have arisen. Genomics is no longer a medical specialty dealing with rare diseases

affecting a small percentage of the population, but is increasingly of relevance to most if not all people and medical specialties (Lea *et al.,* 2010). In order to exploit the full medical as well as health potential of genomics, it is essential to identify the level, gap and needs in genome-based health literacy of various stakeholders ranging from health professionals (i.e. doctors, nurses, dieticians) to politicians, media experts and the population in general. Failure to achieve adequate genome-based health literacy will not only limit the translation of genomic achievements to a health benefit. It may eventually even lead to a misuse of genetics and genomics (McBride *et al.,* 2010).

Target populations including health professionals need to understand the risks and benefits of genome-based health information and related technologies. Some of the challenges in promoting genomic literacy will parallel those previously experienced in the more general health literacy arena. We know that more than one-third of the U.S. adults have limited health literacy, and only about 12% have levels of health literacy skills needed to understand much of today's health information (Kindig *et al.,* 2004). People with limited health literacy have generally low levels of health knowledge, underuse preventive health services, and self-report poorer health. We can therefore expect that a large percentage of the population will face substantial difficulties and barriers in understanding and using genomic information (McBride *et al.,* 2010). A number of challenges deserve special consideration in the context of genome-based health literacy.

First, the internet has become a very important source of health-related information over the past decade. Yet, the USA study recently demonstrated that an internet-based patient portal targeting adults with diabetes was less likely to adequately reach patients with limited health literacy (Sarkar *et al.,* 2010). The internet is an essential source for genomic information and even for guiding decisions to undergo testing such as direct-to-consumer testing. A recent study demonstrated that healthy adults perceived evidence-based genomic information and communications approaches to be helpful for both decisions to test and not to test. But research is essential to ensure that these results generalise to target groups with lower literacy and that are less Internet savvy (Kaphingst *et al.,* 2010). It will be important to avoid technical jargon with which genomics is heavily loaded.

Second, the promise that genomics holds for personalised disease prevention implies a shift from focusing on the treatment of specific diseases to focusing on the treatment of specific patients or even increasingly to focusing on preventing healthy individuals from becoming patients. The concept of numeracy will gain further relevance in genome-based health literacy, especially in the context of multifactorial diseases. Genome-wide association studies (GWAS) are identifying numerous gene variants linked to multifactorial diseases. The effects of single gene variants identified in large-scale genome-wide association studies are mostly very small. Accordingly, the clinical utility of using these gene variants for personalised risk prediction is likely small, as recently demonstrated for type 2 diabetes (Talmud *et al.,* 2010).

As it was mentioned above, the increase in genome-based health information and related technologies is unprecedented and requires a unique level of lifelong learning especially for health professionals. Genomics courses offered to health care professionals and the lay public require continuous update. As it is difficult to foresee medical genomic practice in the years to come, a central aspect of promoting genomic literacy in today's students is the motivation for pursuing lifelong learning (Guttmacher *et al.,* 2007).

Third, genomic information affects an individual's biological network (Johnson *et al.,* 2005). The impact of a genetic test result on family members in the context of direct-to-consumer testing and low penetrance is a novel issue in that context.

Any physician should be able to recognise and interpret familial clustering of diseases such as specific cancers, kidney disease or cardiovascular disorders, in order to refer patients in need to genetic counselling or screening for disease. For example, two of the proposed objectives of Healthy People 2020 are a) to increase the proportion of persons with newly diagnosed colorectal cancer who receive genetic testing to identify Lynch or other familial colorectal cancer syndromes and b) to increase the proportion of women with a family history of breast or ovarian cancer who receive genetic counselling (Amos *et al.,* 2008). Over the past years to promote the assessment of family history for health risk assessment and health promotion, several federal, state, and private organisations have partnered with the Office of the U.S. Surgeon General to raise awareness of both, health providers and general public. Internet-based family history tools are available to assess individual familial risk for several diseases such as heart disease, diabetes, and certain types of cancer (Khoury *et al.,* 2010). The relevance of family history in respect to host genomics and the degree to which it determines a person's susceptibility to a disease is still not fully understood. In case of ocular *Chlamydia trachomatis* for instance, we know that genetic predisposition amounts to 40% of the differences in immune responses to the disease. The risk and the clinical course of infectious diseases appear also to be multifactorial and additionally depend on pathogen's virulence factors as well as environmental factors.

Genomic information can also have potential negative social implications (i.e. insurance coverage; employment status; discrimination) and be linked to positive and negative psychological consequences of a result predictive of severe disease in the absence of symptoms. Both, health professionals and public, needs to be aware of these potential consequences.

Fourth, especially in the Public Health Genomics context not only genomic information, but genome-based health information needs to be addressed when talking about health literacy. That means taking into account genome-environment interactions and integrating all health determinants including genomics into a holistic approach. The highly technology and bioinformatics driven dynamics of genomics as a "moving target" from the Human Genome Project (HGP) to the Personal Genome Project (PGP) is currently

challenging public health research, policy-making and practice in a fundamental way towards a systemic and holistic understanding of the aetiology of diseases or health outcomes ("systems thinking"). It is a new paradigm: translating primarily findings form epigenomics and systems biomedicine we start to understand that, (1) what we call common complex diseases might be a sum of "rare diseases", (2) we move from diseases towards "diseasomes" (disease nodes), (3) we move from risk factors to individual pathways or networks and (4) we move from clinical utility to personal utility. Furthermore, genome-environment interactions change from day to day within an individual. That implies that neither genomics nor the environment can be considered to be 'stable' information. Biological pathways or networks are perpetually interacting with environmental networks such as social networks. Thus, a comprehensive model of future healthcare taking into account integrative genomics alongside with environmental, social and life style factors will become essential to realise the P4 Medicine as the future paradigm of healthcare systems being predictive, personalised, pre-emptive and participatory (Hood *et al.,* 2007). That implies, that in the end also health literacy needs to become personalised.

Stakeholders' roles in the health literacy process

In health care, there are a huge number of parties involved, and they all possess certain interests. These parties attempt to pursue their various (and sometimes conflicting) interests by exerting an influence on health policy. All the involved sides are connected with each other, creating a complex grid of relations that cannot easily be broken down.
Providers comprise both the institutions and the health care professionals. Two main types of the institutions can be distinguished, those that take care of patients (e.g. nursing homes, psychiatric institutions) and those that cure them (e.g. various types of hospitals). Seeking an overview of health care professionals gives an even more intricate picture – for instance: numerous specialties, having an individual practice or working in health centres, self-employed, employed, or self-employed/employed.
Health care *insurers* might have a for-profit or a not-for-profit character, or they might be handling both categories of those receiving insurance. Their coverage can aim at covering the entire population, or target only a distinct portion of it.
Within what could be labelled as industry, we can distinguish pharmaceutical companies (those manufacturing medical appliances included), their role as the managers of the health care process is ever so expanding and thriving (de Gooier, 2007).
All stakeholders should be included in the health literacy actions, not only patients. However, because the majority of attention in health literacy is paid to the patients' perspective, it is also necessary to focus on the changes needed in order to ensure the

successful integration of genome-based information and technologies into the public health system and daily practice.

Focusing on the user

As mentioned above, at the moment, the degree to which the public is involved in genome-based health policy development, at least in certain cases (Hiller *et al.,* 1997), does not seem to be ample and reflects insufficient utilisation of the already instituted mechanisms. Thus, we would like to focus on the changing role of the stakeholder group which is now commonly labelled as "patients". It is important to note that defining the group as "patients" is not objective, because it excludes the representatives of the general public who are not currently ill. With the introduction of genome-based information and technologies into the healthcare we can assume that the term "users of health information" will become more widely used.

The general public representatives need to be provided with novel opportunities which would enable them to participate (pro)actively in their own care. This might be done by targeting behavioural change and developing their self-management skills. It has been proven that those chronic disease programs that successively increase the people's knowledge about their own disease and health are more effective (Herzlinger, 2004). Endeavour such as this requires integrating numerous concepts of knowledge. One of the examples is diabetes type 2, where a patient needs to understand and adopt different levels of knowledge and understanding of all disease-related aspects, for a successful guidance towards lifestyle modification to take place (Ershow, 2009). Aside from being able to understand his or her inherited risk, the person needs to grasp the details on the environmental exposure. For instance, nutrition information can be a very complex set of information to take in – choice of the food group, portion sizes, content of nutrients, energy value and rankings of these values among different foods. The cognitive processing demands of the information related to diabetes education can be overwhelming; therefore it requires the enhancement of competencies, as well as self-sufficiency (Ershow, 2009).

There are on-going efforts to educate the public about genomics, which comes as no surprise, given the tremendous potential that genomics brings. These efforts are mainly founded on the assumption that scientifically literate people tend to feel more positively about science and scientific progress (Zarcadoolas *et al.,* 2006). This kind of relation, however, is not observed in all cases (Pardo & Calvo, 2002).

The concept of a "consumer" entails a relationship that individuals have with their services. Consumer can act as a service user and in that sense have a justifiable interest in provision from his or her personal aspect. There are different approaches when attempting to answer the question on what kind of influence should different

stakeholders exert. There should also be a balance between the individual strivings and desires on one hand and what would be deemed beneficial for the entire community (Callaghan & Wistow, 2006).

Future consumers of health information will have increasingly more means to use this information to their own preference and desired extent – health information is available beyond any past expectations. The speedy developments of new technologies (particularly information and communication technologies) aid in promoting self-care and healthy behaviours. This will inevitably lead to better-informed health decisions, which can result in more suitable demands for health services. One of the major outcomes can be reductions in the overall costs of illness. The fact that almost any health question has an available answer nowadays brings another major implication into the spotlight. The ability of internet and related technologies to provide support to individuals in making informed decisions related to their health may inevitably drive us to the decentralisation of knowledge (Eng *et al.,* 1998). Another question is whether the increasingly available body of knowledge would benefit everyone and equally, however that is an entirely different and extensive issue.

Negative reactions to novel genomic technologies have not consistently been found to be inversely correlated with knowledge and understanding of genomics. People do not necessarily need to understand genomics to trust new technologies, as long as they have a trust in their health care providers or public health agencies (Lanie *et al.,* 2004). Yet, if the understanding and complexity of genome-based health information is too limited or in fact wrong, this can impact on at least three aspects of health-related behaviour. First, people may underuse genetic/genomic counselling when it is indicated. Second, people may not benefit from recent advances in genomics that reach practice in a justified manner (i.e. pharmacogenomic testing such as in the cased of specific cancer diagnosis). Third, subjects lacking adequate understanding of genetic testing and its potential implications are a susceptible target for inappropriate genetic testing such as offered direct-to-consumer over the internet in the absence of solid evidence about the clinical utility. Genome-based health literacy is also of relevance to policy setting (Christensen *et al.,* 2010). Finally genome-based misconceptions can have problematic implications for how people stereotype and think about subgroups of the population (Christensen *et al.,* 2010).

Studies on the status of knowledge related to genes among non-experts in the public in both, the U.S. and various European countries generally showed limited levels of genetic literacy (Lanie *et al.,* 2004; Molster *et al.,* 2009; Christensen *et al.,* 2010; Condit, 2010; Etchegary *et al.,* 2010). Despite the fact that most people are familiar with terms related to genes and genomics and with the multifactorial nature of common diseases - either through biology courses in school or through media reports - the actual knowledge and understanding of the terms is often scientifically incorrect (Lanie *et al.,* 2004; Lea *et al.,*

2010). Much research on the beliefs about the causes of disease and the role of genomics focused on contexts around specific diseases only (Lea *et al.,* 2010).
Quantitative literacy, which is of special relevance to genetic and genomics, is generally lower than other aspects of health literacy. According to the 2003 National Assessment of Adult Literacy only 13% of U.S. adults exhibited proficient levels in quantitative literacy. Twenty two per cent had below basic quantitative literacy and a third had basic quantitative literacy skills according to the National Centre for Education Statistics, 2006. Populations of lower social class or educational level had lower numeracy skills on average as is true for health literacy in general. Little is known about numeracy as it relates specifically to genomics respectively genome-based health information.
Platforms for educating the lay public in genomics, equivalent to the ones described above for health professionals, are still few in number and include those from the National Human Genome Research Institute (cited in 2010) and the Genetic Science Learning Centre, University of Utah (cited in 2010). Those are model programs to improve the genomic literacy of the lay public.

Health literacy and host genomics: implications for subfertility diagnostics

The outcome of host-pathogen interactions can be affected by host genomic factors intrinsic to the patient. Heredity is an important contributor to the risk and the clinical course of infections (Chapman & Hill, 2012). In ocular infections with *Chlamydia trachomatis*, for instance, almost 40% of differences in lymphoproliferative responses to the infection are attributed to genetic variation (Bailey *et al.,* 2009). Moreover, a study by our collaborators has shown that, following a *C. trachomatis* infection, women carrying two or more polymorphisms in PRR genes involved in recognising *C. trachomatis* show more than two-fold higher risk of developing tubal pathology as compared to women with less than two PRR polymorphisms (den Hartog *et al.,* 2006). Subsequent research conducted by our group is part of an overarching framework for the purpose of developing the first immunogenetic trait diagnostic kit for assessing the risk of tubal factor infertility (Branković *et al.,* 2014). The genetic trait diagnostic kit would be dependent on a defined “cut-off” score, enabling better stratification of women with subfertility problems based on their genetic profiles for genes associated with tubal pathology. In cases when female patient’s carriage of risk variants crosses a cut-off threshold, she would be referred by the gynaecologist to other diagnostic procedures (laparoscopy) in order to determine whether tubal damage has been the cause of subfertility. The aim is to reduce the existing high number of invasive procedures performed on women at a low risk of tubal damage, as well as to better target *C. trachomatis*-negative women who are typically not referred to tubal diagnostics in accordance with current protocols.

In order for genome-based diagnostic tools to be successfully implemented into clinical practice, proper understanding genome-based health information is required by health professionals, in this case gynaecologists. Research consistently shows deficiencies in gynaecologists' and other specialists' knowledge and understanding of genome-based information (Baars *et al.,* 2005a; Teng & Spigelman, 2014). Our group's study in preparation on Dutch gynaecologists' and resident physicians' attitudes and knowledge regarding the use of genetic testing in subfertility diagnostics determined that the majority sees the need for additional training on the topic, in order to be able to successfully interpret this information (figure 2). Furthermore, older respondents were more likely to reply that they would require additional training. In addition to that, a Dutch study demonstrated a considerable lack of genetic knowledge in medical students nearing graduation as well (Baars *et al.,* 2005b). Even if adaptations to medical curricula were to take place immediately in order to tackle the problem, we would still witness several generations of insufficiently literate health professionals.

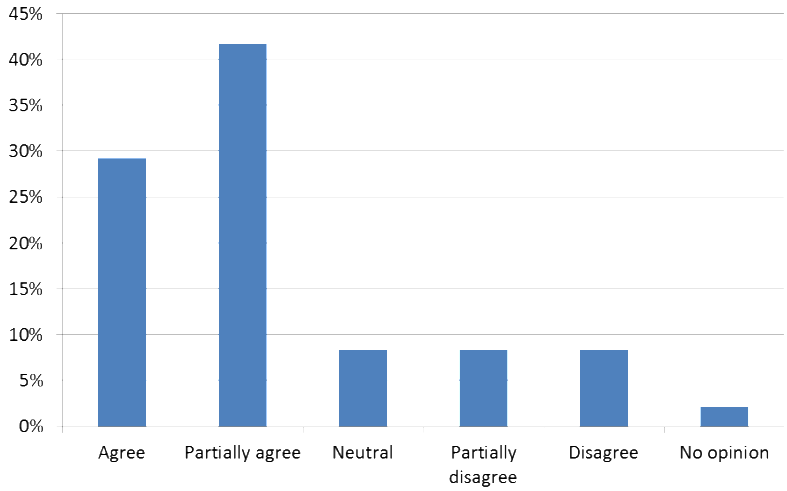

Figure 2. *The responses of gynaecologist and resident physicians to the questionnaire statement: "I would need training if a genetic test was added to subfertility diagnostic procedures" (with permission from Malogajski et al., 2014).*

Future advances in research within host genomics and pharmacogenomics might bring innovations not only to diagnostics, but also therapeutics and other fields of clinical management. At this moment, translational potential of pharmacogenomics findings is promising. Applications of genotype profiling of HIV patients when prescribing antiretroviral therapy are recommended for clinical protocols and already in practice in a

number of countries (Young *et al.,* 2008). These successful examples can serve as a model for tackling the existing challenges in clinical translation and help overcome the current stalemate in facilitating the implementation of knowledge from bench to bedside. Host genomic studies carry the potential to elucidate the most relevant immunogenetic factors in infectious diseases that may lead to more advanced therapeutics. Prescribing immunomodulatory treatments based on a person's genetic profile can significantly improve treatments with minimised side-effects. Developing a therapeutic HPV vaccine would provide novel means of treatment for individuals already infected with hrHPV or suffering from related diseases. The question here would be: in case there is decision to be made on whether to undergo a health intervention based on the patient's genome-based data, would the decision-making remain with the health professional? This would depend on the nature of host genomic application of health technologies. Nevertheless, our current experience with personalised kits such as *BRCA1/2* test attest to the importance of involving a patient in decision-making process on undergoing specific interventions. The role of the patient increases with introduction and expansion of innovative personalised technologies within health care. Constant involvement of patients in decisions affecting their health would be paramount for ensuring better health outcomes in a personalised medicine era (Swan, 2009).

Given how a patient's informed consent is an integral part of genetic testing and counselling, immunogenetic diagnostic trait kits would need to conform to this requirement. We submit that the patient should ideally be informed by the health professional that such mode of obtaining genetic information does not infringe on their privacy rights, given the fact that an immunogenetic diagnostic test would retrieve only limited genome-based information from a small number of selected genes. However, the regulations regarding ethical implications of such health information should be revised periodically, as new input from basic research carries to potential to put these implications in a new context. An example of a polymorphism conferring a Duffy antigen-negative phenotype, *DARC* -46C/C, provides higher resistance to malaria (*Plasmodium vivax* infection) in African-American carriers. However, later studies of this polymorphism revealed a 40% increased likelihood of becoming infected with the HIV-1 virus (He *et al.,* 2008) Evidently, the risks of stigmatisation and discrimination arising from genome-based information (the disclosure of a patient's illness or infection status being a potential infringement of patient rights) may potentially arise with further research. Health professionals should therefore be subject to additional legal bounds regarding confidentiality when dealing with this type of information, so as to prevent future infringements of patient's rights or patient discrimination. Data should be stored on secure intranet devices or appropriately anonymised and encrypted to ensure patient's rights to privacy. Due to the fact that it is unrealistic to expect full proficiency in understanding the impact of genome-based knowledge and risks involved (should they

arise), all the relevant stakeholders need to discuss how to tackle these issues and provide patients with elementary understanding of any such issue, once genome-based technologies achieve their breakthrough into clinical practice. It is for that reason that our group is currently collaborating with the subfertility patient organisation, FREYA, in order to bring stakeholders together and reach a consensus on how to educate and empower patients in the process of implementing the genetic trait-based tool for diagnosing subfertility.

Conclusions

Despite of the relative novelty of this area, health literacy is already an important and established area in public health. However, like the public health system also health literacy is changing in the response to advances in basic sciences and changes in the society in general. Due to rapid and vast developments in genome-based knowledge in the last decades, genome-based health literacy's importance is gradually increasing. However, still most attention is paid to the genetic literacy of the Mendelian disorders, rather than genome-based health literacy linked to a multitude of gene variants and their interaction with the environment (common complex diseases).

It was mentioned that the range of health literacy in general and genome-based health literacy in particular should not be limited to the provision of genome-based information and related technologies to the representatives of the general population. Rather it should help to narrow the information gap among all stakeholders in the Public Health system and get implemented in different tasks of the system (figure 1). Regarding the various stakeholders in public health, it is important to note, that the group labelled as "patients" does no longer reflect the real situation, as it presents all people or individuals as possible patients. Due to the stronger focus on empowering "patients" and paying more attention to the process of health decision-making it would be more appropriate to call the stakeholder group "users of health information".

Advances in genome-based sciences also lead to some challenges. First and foremost, in order for the representatives of the lay public to be able to use genome-based information it is important to on the one hand avoid the intense use of technical jargon, but on the other hand provide the opportunity to understand personalised information enabling informed health decisions. Lifelong learning of both health professionals of different levels and the general public has a crucial role since individual risk assessment may undergo changes as additional gene variants and their interaction with environmental factors are identified. Attention should also be paid to the influence the personalised genome-based information can have on individual's biological network, especially the family.

To be able to overcome the described difficulties as well as the ones that can arise in the future some priorities for future actions in health literacy should be identified. A recent workshop under the auspices of the US National Human Genome Research Institute identified public understanding and use of genomic information as a priority area in communication, behavioural and social science research (McBride *et al.,* 2010).

Efforts to improve genomic literacy should consider Roger's knowledge framework (Smerecnik *et al.,* 2010). It suggests that education and research on genomic knowledge of the public should distinguish between (a) awareness knowledge (knowledge about the existence of an innovation), (b) how-to knowledge (knowledge about the proper use of the innovation), (c) principles knowledge (understanding of the theoretical principles underlying the innovation). According to a recently conducted Dutch study assessing genomic knowledge, a minority of the general population was aware of genetic risk factors of multifactorial diseases, whereas the overall how-to knowledge seemed relatively fair, and principles knowledge was generally poor. Misconceptions about genomics and its influence on disease development were observed (Smerecnik *et al.,* 2010). Previous evidence suggests that adequate decision-making can occur without proper principles knowledge. Yet, principles knowledge diminishes the risk of falsely using perceived knowledge of genetic risk factors in decision-making. Not understanding the principles of genomics has also an impact on the principle of autonomy for decisions regarding medical interventions and genetic testing. Even though many people have a basic understanding of Mendelian inheritance, this knowledge is not sufficient to understand the genetic background of age-related disorders which are mostly the focus of media reports on genomics. Improvement in understanding the principles of genomics of complex diseases is needed for both, health professionals and the lay public.

Improving genomic literacy and making people understand the benefits and limitations of a genetic test may be more challenging in subjects with misconceptions than in subjects knowing that they have no or limited understanding of genomics (Lanie *et al.,* 2004). In the light of studies in the U.S. and Europe that demonstrate that there is still a high level of misconceptions about genomics, it is essential to invest into genetic education at all levels. As mass media are the primary source of genetic information for the lay public, but seemingly also contribute to misconceptions about genomics, the primary education effort should probably be in the training of health care professionals that also still exhibit severe limitations in genetic knowledge. Educating the health professionals will also be fundamental to the persuasion stage in the decisional process related to genetic testing. Furthermore, it is also important that health care professionals consider their clients' knowhow and believes in the area of genomics. It has been suggested that individuals in older age groups, who may well be the subgroup with the greatest potential benefit from advances in the genomics of complex age-related diseases, have lower levels of genetic knowledge and may be more in need for additional and targeted genetic information by

health professionals (Ashida *et al.,* 2010). Prior knowledge and assumptions as well as believes of the target learner in genetic literacy should be considered in all efforts to improve genetic literacy (Lanie *et al.,* 2004).

We still know little about how to measure genomic knowledge: what genomic knowledge is adequate for medical and social decision making in the lay public needs to be further determined (Condit & Shen, 2010). In the U.S. an instrument was developed to understand undergraduate students' genomic knowledge (Bowling *et al.,* 2008). Different assessment tools are needed for different target populations of genomic literacy programs. There is some evidence from a study comparing genomic literacy in different gender and ethnic groups in the US, that even though genomic literacy was comparable and generally poor in all groups, there were some culturally and socially relevant items that seemed to influence specific aspects of genomic knowledge. Genomic knowledge that is retained reflects own and social group interests (Christensen *et al.,* 2010). Another determinant of genomic knowledge and understanding is the family history of genomic diseases (Lea *et al.,* 2010). In addition, research on the effects of mass media on the public understanding of genomics contributes to the discussion, especially given the often overpromising reporting of new genomic findings and the unknown impact of this on knowledge and believes in the public.

Not only do we have to improve our idea on how to best measure genomic knowledge, but even more importantly we need to improve understanding of what is relevant genomic knowledge that people need and use in a profitable manner for decision making (Condit & Shen, 2010).

Although there were initiatives, so far health literacy has rarely been discussed in specific domains of Public Health Genomics. The public health community will lose credibility, if on the one hand public health is promoting health literacy in a value-pluralistic and democratic society and enabling and empowering individuals for decision-making while on the other hand not keeping up with the dynamics of genomics leading to a paradigm shift not only in public health, but also in health literacy. Especially in the Public Health Genomics context not primarily genomic information, but genome-based health information needs to be addressed and provided. That means taking into account genome-environment interactions and integrating all health determinants including genomics into a systemic and holistic approach. Translating findings from epigenomics and systems biomedicine will help to understand that individual biological pathways or networks are permanently interacting with environmental networks such as social networks. Thus, a future healthcare model taking into account integrative genomics alongside with environmental, social and life style factors will become essential (Brand, 2009) to realise the P4 Medicine as the future paradigm of healthcare systems being predictive, personalised, pre-emptive and participatory. In the end, health literacy will also become personalised. Genome-based health literacy would then be challenged by the

question which information is relevant for the individual, for what purpose and at what time during the lifespan. It would be wise to use public health tools and expertise already in place in order to tackle these huge challenges.

Recommendations and future perspectives for host genomic-based health literacy

In regards to the needs to achieve higher standards of specialists' knowledge of genetic and host genomic information, several strategies ought to be undertaken. Firstly, medical education curricula require adaptations that would lead to more salient education of future medical professionals on topics of genetics, host genomics and genome-based technologies. This would ensure not only a higher level of knowledge of future health professionals, but would also empower them in handling genome-based information and technologies, which will inevitably keep changing future medical practice. Furthermore, specialised additional training for medical specialists and general practitioners should be organised, ideally through existing primary and secondary health infrastructures. Given the unforeseeable nature of future genome-based implementations into health care systems, ensuring genomic literacy also needs to be grounded in pursuing life-long learning. The need for increasing genome-based literacy of the patient is an equally important task. Early stakeholder involvement and cross-talk between those stakeholders is essential for quick, effective translation of genome-based personalised medicine approaches such as a subfertility diagnostic kit. Patients are one of the crucial interest parties when introducing such technologies. To accomplish their involvement our group has approached FREYA, the subfertility patient association, so that together with the subfertility patients, clinicians and insurance companies we can determine the optimal strategy for achieving the implementation of genetic trait-based diagnostic tool for subfertility in healthcare systems for the benefit of subfertile patients. The implementation requires increasing health literacy, so as to ensure their full comprehension of the personalised medicine approach and its added value to subfertility diagnostics. The process of implementation would thereafter need to be monitored, to determine how the efforts for increasing genomic literacy do actually reflect on patient's attitudes regarding genome-based technologies. Ensuring the involvement of patients in health-related decision-making would be an essential strategy in achieving better health outcomes in a personalised medicine era.

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DISCUSSION

I don't believe it.
Prove it to me and I still won't believe it.
Douglas Adams

Contents of this chapter

General discussion

Chlamydia trachomatis is the most common sexually transmitted bacterial infection, and it is strongly associated with tubal infertility occurence (Brunham *et al.,* 1985; Sellors *et al.,* 1988; Mabey, 2014). The course of *C. trachomatis* infection varies between individuals - only certain people are successfully infected and only part of those infected develops more severe disease as a result of an uncleared infection and prolonged accompanying inflammation. Infection is frequently asymptomatic, hence actual case numbers are higher than those reported (Morré *et al.,* 2002; Kinnunen *et al.,* 2002; Darville & Hiltke, 2010). Infection with human papillomavirus (HPV) is also very common across human populations. Global prevalence estimates of HPV infection for women range from 2% to 44% (Baseman & Koutsky, 2005). HPV infection with high-risk types induces neoplastic changes in infected tissues, such as cervical epithelium (zur Hausen, 1996). Invasive cervical cancer is one of the most common malignant diseases among women, representing almost 10% of all cancers in the female population. Each year more than 500.000 women are diagnosed with cervical cancer, mostly in developing countries (Torpy *et al.,* 2007). In part I of the present thesis, we presented our research of host genomic factors in *C. trachomatis* and HPV infections. Furthermore, in part II we investigated different phases of translational research of infectious diseases (namely human immunodeficiency virus (HIV), *C. trachomatis* and HPV, from the perspective of biobanking, as well as genomic factors with promising potential for successful translation. Finally, in part III we investigated the role of neglected areas in public health genomics of infectious diseases, gender and health literacy, as the essential components in determining acceptance of and adherence to genomic clinical technologies. After discussing the findings, a final conclusion will be formulated thereafter.

Host genomics in *Chlamydia trachomatis* and human papillomavirus

We have successfully revealed associations of a *NOD1* +32656 T>GG insertion-deletion polymorphism with both susceptibility to and severity of *C. trachomatis* infection in women (**Chapter 1**). Our results show a significantly reduced carriage of the *NOD1* +32656 GG insertion allele in *C. trachomatis*-positive women compared to the *C. trachomatis*-negative women, indicating a potentially protective effect against *C. trachomatis* infections. Presence of *C. trachomatis* IgG antibodies significantly increased in more severe tubal factor infertility (TFI), which confirms the expected relation between past *C. trachomatis* infections and development of tubal pathology. Furthermore, when we compared *C. trachomatis* positive women without symptoms, to *C. trachomatis* positive women with symptoms, to *C. trachomatis* positive women with TFI, we observed an increasing trend in carriage of the *NOD1* GG allele. There was also a significant association

of GG insertion carriage with the occurrence of symptoms during an infection. Based on our findings, in the context of other relevant empirical evidence published on this topic, we proposed a biological hypothesis that the possible effect of the GG insertion variant might be reduced secretion of interferon (IFN)-β, which is known to regulate local immune response to *Chlamydia muridarum* (Nagarajan *et al.,* 2008). This is, however, merely a hypothesis, and further studies on appropriate models would be needed to corroborate it.

In another study (**Chapter 2**) we assessed the impact of polymorphisms in three genes (*VDR* (rs1544410 G>A, rs2228570 C>T), *CYP27B1* (rs10877012 G>T) and *CYP2R1* (rs10741657 G>A)) on susceptibility to *C. trachomatis* infections. He and colleagues (2013) observed in their murine model study that genital chlamydial infection was more severe and prolonged in $VDR^{-/-}$ knock-out mice compared to $VDR^{+/+}$ mice. Inflammatory response also lasted longer in the knock-out mice. In our cohort of 500 *Chlamydia* positive and 1331 *Chlamydia* negative women, we, however, did not observe statistically significant differences between the genotype distributions of the four polymorphisms. For that reason, we assume that *VDR, CYP27B1,* and *CYP2R1* do not play a role in susceptibility to *Chlamydia* infections as they demonstrably do in other diseases. However, due to the pleiotropic nature that genes in vitamin D metabolic pathway play in the immune system, the role of vitamin D ought not be dismissed from the entire clinical course of *Chlamydia* infections (*e.g.* late complications). Follow-up research is therefore required.

Thereby we added to the current knowledge on the effect of immunogenetic factors in infection with *C. trachomatis*. Results of **Chapter 1** are the first report of the role of the *NOD1* +32656 T>GG insertion-deletion polymorphism in the clinical course of this infection. We did not establish associations of vitamin D metabolic pathway genes with *C. trachomatis* infection in **Chapter 2**. All researched polymorphisms from the two studies are presented in table 1. With these studies we contributed to the pool of existing empirical knowledge of host genomic markers in urogenital *Chlamydia* infections. The study by Bailey et al. (2009) has estimated that heritability contributes to differences in proliferative responses to ocular *C. trachomatis* infection with approximately 40%. While we do not have a similar heritability study for urogenital infection with *C. trachomatis*, evidence indicates that host genetic component is quite notable regardless of the tissue infected. A review of host genetic factors in ocular and urogenital *C. trachomatis* infection revealed many shared polymorphisms in pattern recognition receptor (PRR), cytokine and human leukocyte antigen (HLA) genes (Morré *et al.,* 2009). What remains to be further elucidated is which genes are responsible for these responses and how their different variants play a role.

Gene	*Location*	*Polymorphism*
NOD1	7p15-p14	+32656 T>GG, partially identified as rs6958571
NOD2	16q21	1007fs, rs2066847, SNP13, Leu1007fsinsC, 2936insC
VDR	12q13.11	rs1544410 G>A
		rs2228570 C>T
CYP2R1	11p15.2	rs10741657 G>A
CYP27B1	12q14.1	rs10877012; -1260 G>T

Table 1. *Polymorphisms analysed in original research articles within the scope of this thesis*

In **Chapter 3**, we summarised a comprehensive list of all studied immunogenetic polymorphisms pertinent to HPV infection and cervical cancer. We included genes coding for cytokines, chemokines, immune receptors and other related to immune response and regulation, excluding HLA genes, KIR genes and methylation markers. In it, we provide all the reported significant and non-significant findings within the tables (**Chapter 3**, tables 2-4). In **Chapter 5**, we outlined HLA genes and methylation markers that have the potential for successful translation into health care applications for HPV and cervical cancer management.

Being the first points of contact in recognising the pathogen and eliciting an effective immune response to it, pattern recognition receptors (PRRs) have a critical role in defence and maintenance of organism's uncompromised integrity. Moreover, the signalling pathways of these receptor molecules converge, eventually triggering the same intracellular cascade of events which will result in the activation of nuclear factor-kappaB (NF-κB). This transcription factor regulates the expression of a range of pro-inflammatory cytokine genes (Kawai & Akira, 2007). Therefore, by recognising pathogen-associated molecular patterns (PAMPs), PRRs induce inflammation and directed adaptive immune response at the site of the infection.

Urogenital *C. trachomatis* infection is known to induce damage to the reproductive system of women. The infection can often be cleared by itself or easily treatable when it remains confined to the lower genital tract. However, uncleared or repeated infections can ascend to the upper genital tract, causing augmented inflammatory responses in some women. Tubal occlusion and periadnexal adhesions are some of the late complications that may result in subfertility or infertility (Morré *et al.,* 2002; Darville & Hiltke *et al.,* 2010; Haggerty *et al.,* 2010). Evaluation of the causal link between lower genital tract infection with *C. trachomatis* and tubal infertility is a challenging task, due to the fact that this is a

"silent" complication, often diagnosed years after the infection initially occurred (Land *et al.,* 2009).

Although evidence exists that bacterial virulence factors affect the level of risk in *C. trachomatis* infection (Naher *et al.,* 1991; van Duynhoven *et al.,* 1998; Morré *et al.,* 2000), host genomic factors are likely a stronger determining component, as suggested by more recent studies (den Hartog *et al.,* 2006; Bailey *et al.,* 2009; den Hartog *et al.,* 2009; Ouburg *et al.,* 2009; Jiang *et al.,* 2012; Lal *et al.,* 2013; Al-Kuhlani *et al.,* 2014). Most studies have assessed variation solely in either the host or pathogen genome, whereas it is likely that the outcome of exposure to an infectious agent reflects the interaction between specific human and pathogen genotypes. The resulting co-evolution might also entail pathogen's adaptation to specific host factors that are present in some subpopulations of humans. These processes may in some instances account for geographical distribution observed in some pathogen strains (Chapman & Hill, 2012).

The degree of pathogen diversity may directly influence the success of infectious disease genome-wide association studies (GWASs). For example, host genomic polymorphism might exert a greater influence on inter-individual variation in susceptibility in the setting of the unusually low levels of *Mycobacterium leprae* genomic diversity. Factors such as the dose and route of infection — as well as the possibility of co-infection with multiple pathogen strains — may also be involved in determining the outcome of the host–pathogen interaction (Chapman & Hill, 2012). These factors are, however, not easy to account for in genetic association studies. The degree to which host and pathogen genetic factors and environment contribute to clinical phenotypes and mutually interact is still to be explained (Chapman & Hill, 2012).

Translational efforts – from bench and *almost* to bedside?

Biobanks are invaluable resources in genomic research of both the infectious diseases and their hosts. In **Chapter 4** we examined the role of biobanks in basic research of infectious disease genomics, as well as the relevance and applicability of biobanks in the translation of impending knowledge and the clinical uptake of knowledge of infectious diseases. Our research identified potential fields of interaction between infectious disease genomics and biobanks, in line with global trends in the integration of genome-based knowledge into clinical practice. Furthermore, it examines various networks and biobanks that specialise in infectious diseases (including human immunodeficiency virus (HIV), HPV and *C. trachomatis*), as well as examples of successful research and clinical uptake stemming from biobanks. Our article also outlines key issues with respect to data privacy in infectious disease genomics, as well as the utility of adequately designed and maintained electronic health records. We maintain that the public should be able to easily access a clear and detailed outline of regulations and procedures for sample and data utilisation by

academic or commercial investigators, and also should be able to understand the precise roles of relevant governing bodies. This would ultimately facilitate uptake by researchers and clinics. As a result of the efforts and resources invested by several networks and consortia, there is an increasing awareness of the prospective uses of biobanks in advancing infectious disease genomic research, diagnostics and their clinical management. A clear overview of the usage of existing infectious disease biobanks is lacking in present literature, and we maintain that this information should be readily accessible to the public, along with clear regulatory and procedural guidelines for utilisation of samples and data. This would ultimately facilitate the currently insufficient uptake by researchers and clinics. Several biobanks have, however, already set high standards in terms of instating appropriate regulation as well as enabling successful translation into clinical setting and can therefore serve as a model to other biobanks. In recent years, efforts and resources that have been invested in biobanking networks and consortia have surged. As a result, there is a higher awareness of the multitude of ways in which biobanking can advance basic research, diagnostics and - most importantly – the clinical management of infectious disease. These advances will ensure that research in biobank-based infectious disease continues to progress.

In **Chapter 5** we reviewed the state-of-the-art basic host genomic and genetic findings' translational potential for HIV, *C. trachomatis*, and HPV into applications in public health, especially in diagnosis, treatment, and prevention of complications of these infectious diseases. To our knowledge, this is the first review on the translational potential of such findings for HIV, *C. trachomatis*, and HPV into applications in public health and in diagnostics, treatment, and prevention of late complications of these infectious diseases. We found scarce examples of the current application of genomic and genetic findings, in pharmacogenomics, and we found examples of genomic information with a promise of translation in the near future. In the review, we did not focus on analytic validity, clinical validity, and clinical utility and other criteria generally considered to be the most important factors in evaluation of the genetic/genomic applications (Teutsch *et al.,* 2009). Since there are still no ready-for-market applications, the aforementioned criteria could not be considered; therefore we focus on earlier steps of the translation trajectory. We examined the promising examples of translation of the discovery into a possible application.

Subfertility poses an enormous burden on healthcare and society throughout the world. Worldwide, 15% of couples trying to conceive suffer from subfertility (Evers, 2002; Broeze *et al.,* 2010). One of the major causes of female subfertility is tubal pathology (TP) (Evers, 2002), and *C. trachomatis* is the single most common cause for infertility. If left untreated, it may lead to ectopic pregnancy, tubal pathology, and ultimately infertility. The cost associated with subfertility is high, as it requires tubal surgery and in vitro fertilisation (IVF). Currently, CT IgG serology is used to assess the risk of *Chlamydia*-associated TP in

subfertile women (20%) (Broeze *et al.,* 2011). *Chlamydia* serology has limited sensitivity and specificity and the predictive value is poor thus, many women undergo additional diagnostic procedures while not needed (40–45%) or do not get intervention while needed (19%). Laparoscopy is widely used to assess the risk of TP in women positive for *Chlamydia* IgG. This procedure is invasive and expensive (on average 3000 EUR including additional costs) and requires general anaesthesia. Furthermore, it holds a 1.5% risk of surgical complications (e.g., bleeding, infection, or worse). Therefore it is crucial to develop a companion diagnostic to improve the assessment of risk of TP in *C. trachomatis*-positive and negative women. By doing so, one is able to prevent invasive procedures in patients without TP and reduce both the cost and the psychological burden associated with laparoscopy. This companion diagnostic should merge serology, taking into account serological positivity and titres and considering new serological responses (*e.g.* pgp3) (Wills et al 2009) and add the predictive value of host genetic markers involved, for example, related to the innate immune response to pathogens. The genetic trait kit should consist of a series of markers with a so-called 'SNP load' and would require defining a specific "cut-off" score for carriage of protective or risk-increasing variants of polymorphisms. It would enable better stratification of women with subfertility problems based on their genetic profiles for genes associated with tubal pathology. A number of studies conducted by our group, our collaborators, but also other researchers, has demonstrated that host genomic markers appear to be the most adequate indicators of late complications in women with *C. trachomatis* infection (Barr *et al.,* 2005; den Hartog *et al.,* 2006; Bailey *et al.,* 2009; den Hartog *et al.,* 2009; Ouburg *et al.,* 2009; Jiang *et al.,* 2012; Lal *et al.,* 2013; Al-Kuhlani *et al.,* 2014). Future studies should be directed at performing studies in larger cohorts to access the true clinical potential of this approach.

Epigenetic alterations, mainly aberrant DNA methylation of tumour suppressor genes, have been receiving substantial attention over the recent years, in the context of cervical lesion assessment. Increasing levels of alterations in host cell DNA methylation have been consistently observed with each successive stage of cervical lesion (Steenbergen *et al.,* 2014). In **Chapter 5**, we highlighted methylation patterns in *MAL* and *CADM1* genes as optimal markers for the development of a cervical lesion triage test (Overmeer *et al.,* 2011). In their review of best prospective biomarkers for cervical cancer diagnosis, Litjens and co-authors (Litjens *et al.,* 2013) also concluded in their review that *MAL* and *CADM1* methylation levels bring the highest predictive value, but added p16INK-4a/Ki-67 dual immunostaining and viral integration to the proposed set of markers. Steenbergen and co-authors (2014) also propose *MAL* and *CADM1*, as well as *mir-124-2*. A caveat here is that only a few human gene methylation markers have been sufficiently researched as methylation marker panels for their potential use in triage. *MAL-CADM1* panel has successfully reached validation framework phases 3 and 4 (Hesselink *et al.,* 2011). Similar progress has been achieved for *MAL-mir-124-2* panel (Hesselink *et al.,* 2014).

Tests for hrHPV DNA do appear to have a shortcoming, in terms of conferring 2–4% lower specificity for CIN2+ compared to cytology. However, in order to avoid overdiagnosis (due to its ability to additionally detect transient HPV infections), algorithmic corrections can be added (Steenbergen *et al.,* 2014). Research confirms that HPV-based screening approaches are advantageous to Pap smear tests in protecting against cervical cancer (Ronco *et al.,* 2013). Self-testing with hrHPV test kits was also found to be very manageable for at-home setting and is a point of further interest (as reviewed in Snijders *et al.,* 2013).

Gender, socio-cultural environment, intersectionality and personalised medicine

In addition to biological sex differences (Doyal, 2001), sociocultural gender differences, as well as their various interactions, can help predict HPV risk and risk behaviour, healthcare access and screening rates, and consequences, as we have explained in **Chapter 6**. The 'omic' applications within the past decade led to the development of revolutionary preventive and screening tools. In 2006, a recombinant quadrivalent vaccine, Gardasil, for the prevention of infections by HPV types 6, 11, 16, 18 was approved for use by the U.S. Food and Drug Administration (FDA). Approval of the bivalent vaccine, Cervarix, targeting HPV types 16 and 18 followed suit (Villa, 2011). Although HPV DNA testing is perceived as an added value and is not expected to reduce adherence to Pap screening (McCree & Dempsey, 2005), informing women of their HPV status has both benefits and risks. A positive HPV test result may promote safer sexual practices, reinforce adherence to Pap screening and follow-up, or engage in preventive actions to reduce the risk of cervical cancer. On the other hand, it may lead to negative psychological side effects, namely stigma, blame and shame (Perrin *et al.,* 2006). Lack of knowledge on HPV infection gap is present across a broad age spectrum – in women aged 18-49 (Hutson *et al.,* 2011). It even persists among University educated women of certain ethnic and religious backgrounds, and it negatively influences their participation rates for HPV vaccination (Gerend & Sheperd, 2011). Women of certain ethnicities (*e.g.* Singaporean women) do exhibit high acceptability of the vaccine, despite low awareness and inadequate knowledge (Pitts *et al.,* 2009). Lower vaccine initiation was also associated with having parents with low incomes, having public insurance, and having fewer sexual partners (Tiro *et al.,* 2012). On the issue of national vaccination programmes, hardly any debate took place on whether the vaccination should include boys as well. Countries that implemented HPV vaccination targeted only young women (Hull & Caplan, 2009). Vaccination programmes were based on the presumption that there is a defined age at which all girls are negative for HPV, thereby failing to account for the girls already exposed to HPV, either via non-sexual contact or as a result of childhood sexual abuse (Godfrey, 2007). A more conscientious approach in stratifying risk subgroups and identifying the intersection where all relevant

factors interact to produce a unique mosaic, determining individual's risk and health outcomes.

It is our opinion that the intersectional approach to biomedical sex and sociocultural gender research and clinical practice could be beneficial for advancing personalised medicine. Using pre-selected categories (*e.g.* men and women) and treating them as homogenous and detached from other variables is often counterproductive (Hankivsky, 2012). Taking gender into account in the clinical management of HPV and related diseases is a necessary step, however it ought not be the final step. Gaining insight into what happens *within* and *between* different groups takes screening, diagnostics and treatment beyond isolated subgroup analyses. Current clinical practice assumes classifications such as gender or SES as independent categories. However, unequal social relations are incorporated in broad systems of historical inequalities which intersect, overlap, and reinforce each other to shape a person's health status. As regards HPV prevention, screening, and treatment, we find that many subgroups of women are underserved such as women living in rural areas, women who have sex with women (WSW), older women, or women with low health literacy levels. Often those at higher risk of cervical cancer tend to show lower awareness and knowledge regarding the HPV virus and HPV vaccine. In acquisition and transmission of HPV, gender relations are essential because of: physical and biological aspects; structural factors such as access to healthcare; social differences between men and women and unique interplays of social factors for each subgroup. Being vigilant of different potential strategies for prevention of HPV and a conceptualisation of gender as a relational and intersectional concept in which the health of men and women impact each other is of major importance. Research on the role of host genetic susceptibilities may yield more insights on the risk factors in the near future.). By incorporating sex and gender analysis into public health genomics explaining differences in a number of diseases between men or women would be more easily achieved (Verdonk & Klinge, 2012).

Health literacy and advancement of knowledge

Chapter 7 outlines the relevance of health literacy in host genomic applications. The chapter presents issues pertinent to acceptance and implementation of genome-based health technologies, such as host trait diagnostic tools into the clinical setting. In order for genome-based diagnostic tools to be successfully implemented into clinical practice, proper understanding of genome-based health information is required of health professionals, in this case gynaecologists. Research consistently shows deficiencies in gynaecologists' and other specialists' knowledge and understanding of genome-based information (Baars *et al.,* 2005a; Salm *et al.,* 2014; Teng & Spigelman *et al.,* 2014). Our group's study in preparation on Dutch gynaecologists' and resident physicians' attitudes

and knowledge regarding the use of genetic testing in subfertility diagnostics determined that the majority sees the need for additional training on the topic, in order to be able to successfully interpret this information (**Chapter 7**, figure 2). Furthermore, older respondents were more likely to reply that they would require additional training. In addition to that, a Dutch study demonstrated a considerable lack of genetic knowledge in medical students nearing graduation as well (Baars *et al.,* 2005b). Even if adaptations to medical curricula were to take place immediately in order to tackle the problem, we would still witness several generations of insufficiently literate health professionals.

In regards to the needs to achieve higher standards of specialists' knowledge of genetic and host genomic information, several strategies ought to be undertaken. Firstly, medical education curricula require adaptations that would lead to more salient education of future medical professionals on topics of genetics, host genomics and genome-based technologies. This would ensure not only a higher level of knowledge of future health professionals, but would also empower them in handling genome-based information and technologies, which will inevitably keep changing future medical practice. Furthermore, specialised additional training for medical specialists and general practitioners should be organised, ideally through existing primary and secondary health infrastructures. Given the unforeseeable nature of future genome-based implementations into health care systems, ensuring genomic literacy also needs to be grounded in pursuing life-long learning.

The need for increasing genome-based literacy of the patient is an equally important task. Early stakeholder involvement and cross-talk between those stakeholders is essential for quick, effective translation of genome-based personalised medicine approaches such as a subfertility diagnostic kit. Patients are one of the crucial interest parties when introducing such technologies. To accomplish their involvement our group has approached FREYA, the subfertility patient association, so that together with the subfertility patients, clinicians and insurance companies we can determine the optimal strategy for achieving the implementation of genetic trait-based diagnostic tool for subfertility in healthcare systems for the benefit of subfertile patients. The implementation requires increasing health literacy, so as to ensure their full comprehension of the personalised medicine approach and its added value to subfertility diagnostics. The process of implementation would thereafter need to be monitored, to determine how the efforts for increasing genomic literacy do actually reflect on patient's attitudes regarding genome-based technologies. Ensuring the involvement of patients in health-related decision-making would be an essential strategy in achieving better health outcomes in a personalised medicine era.

As we have also stated in **Chapter 6**, low health literacy levels are solid predictors of women's attendance to cervical cancer screening (Peterson *et al.,* 2008; El-Hammasi *et al.,* 2009; Leung & Leung, 2010). Implementation of a triage tool based on assessing genome-based data brings additional issues with regard to the patient's required level of

comprehension and acceptance of these applications. It is therefore imperative to involve patients as early as possible when implementing the triage tool and to monitor their attitudes and experiences when presented with genome-based information relevant for comprehending the tool and diagnostic interpretation.

Public health genomics of *Chlamydia trachomatis* and human papillomavirus

Public health policy has traditionally had an important role in tackling such threat through established measures of prevention, mostly by controlling social and other environmental determinants of health and through vaccination. With the recent advances in public health genomics, public health moved its focus from a "one size fits all" approach in health promotion and prevention activities to targeting populations and subpopulations with defined genetic risks and developed its unique role, translation of genome-based knowledge and technologies into public health policy and practice, and its integration across disciplines. The antiquated character of the "one size fits all" paradigm in infectious disease management manifests itself in the failure to recognise inherent differences in both the host and the pathogen. Scientific developments in basic research and the development of public health genomics have changed many paradigms regarding infectious diseases. Indeed, the recent evidence of genetic factors in the pathogenesis of infectious diseases transformed the view of such diseases from strictly pathogen-centric to the one incorporating host genetic determinants that modulate immune response. Though research in the field of genetic susceptibility to infectious diseases started more than 50 years ago, recent progress in genomics led to the characterisation of molecular biomarkers and pathways as understanding of infectious diseases explains the individual variation in susceptibility to an infection as well as the clinical course of the infection by pathogen related factors, environmental factors, and genetic differences. Therefore, establishing implementation of host genomic factors into the clinical setting and maintaining a continuous input of new evidence-based knowledge would lead to advances in infectious disease management. The Bellagio model which represents the "enterprise" of public health genomics can serve as a basis for conceptualising the translation of knowledge from host-pathogen genomic research into clinical applications, policy and education (figure 1).

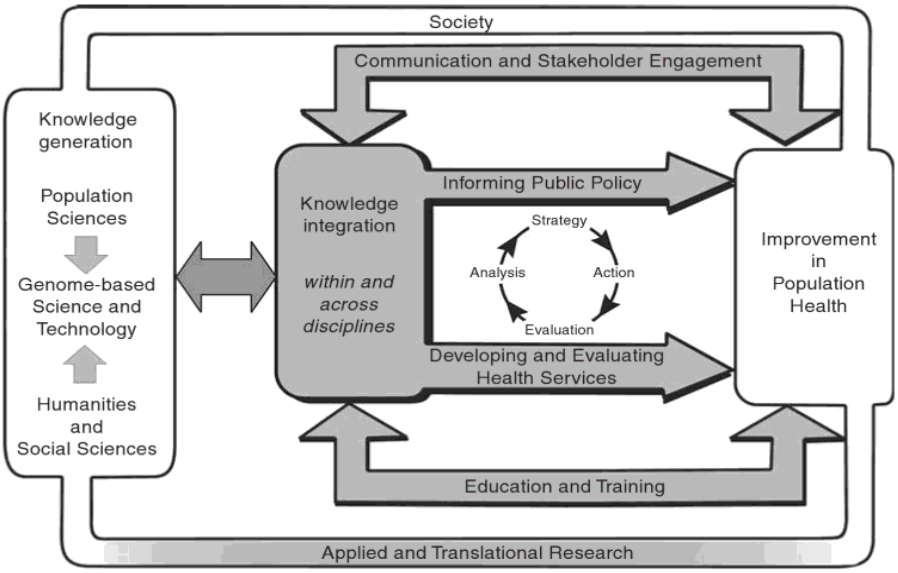

***Figure 1**. Strategy for the effective translation of genome-based knowledge. Adapted from Willard & Ginsburg, 2009.*

Epigenomics is a rapidly advancing field that contributes greatly to our understanding of diseases. Epigenomic alterations are essential for HPV-driven progression of neoplastic changes in cervical epithelial cells (Steenbergen *et al.,* 2014). Another promising aspect of epigenomics for future clinical management of infectious diseases is the (theoretical) ability to modify the biochemical changes occurring, thereby restoring gene expression. From the perspective of public health genomics of infectious diseases, taking into account epigenomic information ought to enhance the disease model and advance successful translation into prevention, diagnostics and therapy.

Furthermore, incorporating neglected socio-environmental stratifying elements into public health genomics models would be imperative. Gender is not only a predictor of health outcomes, it is a determining factor in acceptance of and adherence to clinical technologies, as we have argued in **Chapter 6**. There is a low awareness of the contribution of gender in health outcomes, even nowadays among medical community (Verdonk *et al.,* 2012). These factors are modifiable by effective interventions, therefore it is essential to raise awareness of them. Basic research also needs to be conducted on segregated genders, even in case of cell cultures, and gender should always be reported in biomedical studies (http://genderedinnovations.stanford.edu/terms/gender.html). And lastly, the translation requires increasing health literacy, so as to ensure their full comprehension of the personalised medicine approach and its added value to health care.

There were various education programmes developed within the field of public health genomics in order to promote and enhance genomic literacy in specialists and users (patients) (Burton, 2003; Stewart *et al.,* 2007).

Limitations

Even though there is sufficient research available which implicates the role of host genomic factors in infections with C. trachomatis and HPV, the precise effects and interactions with other components of the genome, epigenome, transcriptome, and metabolome are not fully understood. Immune responses to infections exhibit in most cases a polygenic character - each gene affecting the response accounts with modest contribution to the sum heritability. Introduction of other factors, such as socio-cultural environment, only add to the complexity at hand. Moreover, the role of gene variants that can affect a disease may change in presence of different environmental factors. In **Chapters 1** and **2**, we researched limited number of polymorphisms. For the purpose of analysing their potential synergisms with other variants, more polymorphisms of sufficient frequencies ought to be added to follow-up studies. In **Chapter 3**, we summarised a comprehensive list of all studied immunogenetic polymorphisms in HPV infection and cervical cancer. In our literature selection, we excluded HLA genes and KIR genes, due to the number of studies available. A separate comprehensive review would be in order for those gene groups. In **Chapter 6**, we retrieved studies from three leading journals in the field of gender medicine – *Women & Health*, *Gender Medicine* and *The Journal of Women's Health*. Additional searches were performed, but without a thorough literature search of the kind we applied for retrieving the studies from the three journals mentioned. Although we obtained a considerable number of studies on the researched topics (**Chapter 6**, table 1), omission of relevant findings published elsewhere is a possibility.

Implications for health care and future research

Despite the fact that the landmark study of Gambian twin pairs by Bailey and co-authors (2009) corroborated that host genetic component majorly contributes to the difference in immune response to ocular *C. trachomatis*, we find it important that a replication study is conducted for the purpose of determining its exact contribution in urogenital infection. The major obstacle in accomplishing such research would be to obtain a sufficient number of infected twin pairs, which makes it highly unlikely that such a study can be realised. Although we would not expect the findings of such study to majorly deviate from those observed for ocular infection, it would help our understanding of possible tissue-specific differences and genetic predisposition. Furthermore, large-scale studies attempting to

elucidate precisely which genes and polymorphisms are responsible for differences in susceptibility to and severity of the urogenital infection by *C. trachomatis* would enable the identification of clinically relevant traits that would be the basis of an innovative diagnostic tool for the purpose of improving management of patients. Ongoing work by our group and collaborators is already contributing to these efforts and we hope to successfully map out all relevant factors, determine their functionality, and complete the translation trajectory to the field of clinical diagnostics. Additionally, identifying rare variants in PRR genes represents another promising field that could bring us more insight into the actual susceptibility factors to *C. trachomatis* and HPV. Smirnova et al. have already concluded that rare variants in *TLR4* affect susceptibility to meningococcal disease (Smirnova *et al.,* 2003). The character and scope of interactions between rare and common variants is not yet explained and most likely is dependent on the disease, host and affected tissue. We already know of cases where the penetrance of rare variants is under heavy modification of common alleles, for instance in cystic fibrosis (Gu *et al.,* 2009). In order to identify immunogenetic factors – rare and common – with strongest predictive value, next-generation sequencing and genome-wide association studies (GWAS) are a favourable alternative and can be used next to the candidate gene approach. Roberts and colleagues (2014) were the first to use the GWAS approach to screen for pathway-wide genomic differences comparing cases of scarring trachoma with controls. They used Pathway of Distinction Analysis (PODA) to test for associations. Another new innovative approach (Su *et al.,* 2014) uses an advanced recombinant inbred mouse strain set to identify sets of genes associated with disease severity phenotypes with special attention to genes associated with upper genital tract complications. This innovative state-of-the-art tool is expected to advance human gene discovery and identify novel genes linked to the susceptibility to and severity of *C. trachomatis* disease.

Testing for epigenetic changes in gynaecological setting has the potential to improve the identification of hrHPV-positive women in late stages of cervical neoplasia. However, there is also the prospect of personalised therapeutic innovations. The fact that methylation is in essence a reversible reaction is already being utilised for the research of demethylating drugs for different types of neoplasia. So far, the development of these drugs is still in its infancy and is hindered by severe side-effects (Gnyszka *et al.,* 2013). It is nevertheless conceivable that future therapeutic strategies might enable a limited, locally targeted delivery of such drugs to affected tissues.

Most genome-based studies assess variation primarily by focusing on either the host or the pathogen, whereas the course of infection is likely to depend on dynamic interactions between specific human and pathogen genotypes (Chapman & Hill, 2012). In that respect, an applicable integrative approach would aid in handling these multi-level data, 'from basic molecular interactions to organism responses' (Forst, 2006). The still-fledgling field of host-pathogen system biology examines the potential models and perspectives for

integrating such data. Future advances in this field would enable better addition of all the relevant information into one model and examining the complex interactions at hand. Forst (2006) gives an exemplary host-pathogen network model for *Chlamydia psittaci* infection, explaining the connectivity of host's and pathogen's tryptophan biosynthesis network and how its components modulate the course of the infection. The model also explains how the pathogen intercepts specific metabolites and hijacks host's tryptophan depletion cascade, thereby securing its growth and persistence. Extrapolating the knowledge on similar pathogens (*e.g. C. trachomatis*) would create possibilities for developing new therapeutics.

The degree to which host genomic variation attributes to a person's risk and clinical course of infectious disease is ultimately contingent upon the outcome of interactions with other health determinants (Alcaïs *et al.,* 2009). In addition to that, however, the more the pathogen is genetically diverse, the less easy it is to determine the associations of host genomic factors with the disease. Therefore, the prospective success of host-pathogen research in explaining the nature of host responses and health outcomes might to a certain degree be influenced by the genetic diversity of infection in question (Chapman & Hill, 2012). Having that in mind, major breakthroughs in clinical risk prediction based on host genomic translation might not be achieved in the most immediate future. Alternatively, examples of successful clinical implementation could perhaps be accomplished for certain infectious diseases more easily than others. Also, applications of immunogenetic research carry the potential for progress in the field of next generation therapeutics, such as immunomodulatory drug development, hence the pace of advancements in clinical translation is expected to differ between diagnostics and treatment as well.

Conclusion

Studies of host genomics are a promising strategy to determine the nature of risk factors in *C. trachomatis* and HPV infections, as well as their sequelae. This basic research can provide the basis for genomic applications into health care. These applications would involve genome-based diagnostic tools with improved predictive value and more adequate triage. Other clinical implications of this research pertain to the development of new prevention methods and therapies, such as immunomodulatory drugs for individualised treatment of specific deficiencies in gene products mediating the immune reponse and inflammation. Further innovation in vaccine design and serotherapy also depend on understanding of host-pathogen interaction. With improved understanding of immune and inflammatory processes underlying the course of infection it should be easier to interpret epidemiological findings. Hence, we should expect more success in stratifying different groups of patients, based not only on duration of the infection, but also their susceptibility and severity markers. Empirical findings of host genomic factors demand responsible and effective translation into clinical setting and policy. Public health genomics is a field of research that comprises responsible and effective translation of genomic research results into health care applications. The field is redefining health paradigms by recognising the necessity of taking into consideration all individual characteristics and their interactions between genomic, biological and environmental determinants in order to fully comprehend disease aetiology and provide successful prevention, screening and treatment. The prerequisite for the advancement of personalised medicine approaches in infectious diseases is ensuring continuous research of host's and pathogen's factors, providing more robust efforts for translation of obtained knowledge and incorporating the neglected factors (such as gender and health literacy) more decisively into public health genomics models.

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Summary

This thesis presents research and discussion of several aspects in public health genomics of infectious diseases for *Chlamydia trachomatis* and human papillomavirus (HPV). With over 100 million new infections annually, C. trachomatis represents the most ubiquitous sexually transmitted bacterial infection. Infection with HPV is also very common across human populations, with global prevalence estimates for women ranging from 2% to 44%. HPV infection with high-risk types induces neoplastic transformation of infected cells. Cervical cancer is one of the most common malignant diseases among women, representing almost 10% of all cancers in the female population. In this thesis, studies on host genomic factors in *C. trachomatis* and HPV infections are presented, as well as prominent efforts to translate the obtained empirical knowledge. There are on-going efforts to effectively translate host genomic findings into diagnostics, prevention and therapeutics, for the purpose of improving clinical management of patients and population health. Furthermore, contribution of gender as a socio-cultural factor and health-literacy in regard to public health genomics of infectious diseases has been researched and elaborated on. Gender is a strong factor that affects health outcomes, yet mainstreaming of gender within health care and medical education is at this point still insufficient. Also, research consistently shows deficiencies in gynaecologists' and other specialists' knowledge and understanding of genome-based information.

Below, the content of the three parts and their respective chapters is summarised.

Part I of the thesis comprises research on host genomic (immunogenetic) variants in infections with *C. trachomatis* and HPV. In **Chapter 1**, we have investigated the role of functional polymorphisms in NOD1 and NOD2 receptors, we have successfully revealed associations of a *NOD1* +32656 T>GG insertion-deletion polymorphism with both susceptibility to and severity of *C. trachomatis* infection in women. Our results show a significantly reduced carriage of the *NOD1* +32656 GG insertion allele in *C. trachomatis*-positive women compared to the *C. trachomatis*-negative women, indicating a potentially protective effect against *C. trachomatis* infections. Presence of *C. trachomatis* IgG antibodies significantly increased in more severe tubal factor infertility (TFI), which confirms the expected relation between past *C. trachomatis* infections and development of tubal pathology. Furthermore, when we compared *C. trachomatis* positive women without symptoms, to *C. trachomatis* positive women with symptoms, to *C. trachomatis* positive women with TFI, we observed an increasing, statistically significant, trend in carriage of the *NOD1* GG allele [p 0.0003].
The potential impact of polymorphisms in three genes (*VDR* (rs1544410 G>A, rs2228570 C>T), *CYP27B1* (rs10877012 G>T) and *CYP2R1* (rs10741657 G>A)) on susceptibility to *C.*

trachomatis infections was assessed in **Chapter 2**. Gene variations in vitamin D metabolic pathway have been demonstrated to mediate responses to infections. In our cohort of Dutch Caucasian women, we, however, did not observe statistically significant differences between the genotype distributions of the four polymorphisms. For that reason, we assume that *VDR, CYP27B1,* and *CYP2R1* do not play a role in susceptibility to *Chlamydia* infections as they demonstrably do in other diseases. However, genes in vitamin D pathway exhibit a pleiotropic role in the immune system, therefore the role of vitamin D ought not be dismissed for the entire clinical course of *Chlamydia* infections and should be researched further.

Chapter 3 provides a comprehensive summary of all studied immunogenetic polymorphisms pertinent to HPV infection and cervical cancer. We included genes coding for cytokines, chemokines, immune receptors and other related to immune response and regulation.

Part II of this thesis focuses on the starting and the finishing step of translation of knowledge from bench to bedside. **Chapter 4** examines the role of biobanks in research of infectious disease genomics and the potential for using the knowledge in ensuring its effective clinical uptake and application. This chapter also outlines prominent examples of successful usage of biobanks in the field of host genomics of infectious diseases (specifically human immunodeficiency virus (HIV), HPV and *C. trachomatis*). Also, main issues regarding data privacy and maintaining electronic health records in the context of infectious disease genomics research are provided. Finally, recommendations for governance of infectious disease biobanks and regulatory procedures for sample and data utilisation are provided.

In **Chapter 5** we reviewed the state-of-the-art basic host genomic and genetic findings' translational potential for human immunodeficiency virus (HIV), *C. trachomatis*, and HPV into applications in public health, especially in diagnosis, treatment, and prevention of complications of these infectious diseases. To our knowledge, this is the first review on the translational potential of such findings for HIV, *C. trachomatis* and HPV into applications in public health and in diagnostics, treatment, and prevention of late complications of these infectious diseases. We found scarce examples of the current application of genomic and genetic findings, in pharmacogenomics, and we found examples of genomic information with a promise of translation in the near future.

Part III emphasises two neglected areas in infectious disease research and clinical management. Research has shown that gender as a socio-cultural factor can help predict HPV risk and risk behaviour, healthcare access and screening rates, and health consequences, as we outlined in **Chapter 6**. The study explains how gender is not only a predictor of health outcomes - it is a determining factor in acceptance of and adherence to clinical technologies, such as HPV DNA testing and HPV vaccine. **Chapter 7** outlines the relevance of health literacy in host genomic applications. The chapter presents issues

pertinent to acceptance and implementation of genome-based health technologies, such as host trait diagnostic tools into the clinical setting. In order for genome-based diagnostic tools to be successfully implemented into clinical practice, proper understanding of genome-based health information is required of health professionals. Research consistently shows deficiencies in gynaecologists' and other specialists' knowledge and understanding of genome-based information. Host genomic research will be changing clinical practices in the near future, hence it is of crucial importance that health professionals as well as patients and users are capable of interpreting this information correctly. In the chapter, our group's research on gynaecologists' and physicians' knowledge and attitudes towards implementation of genome based technologies and efforts in involving health professionals and patient organisations in implementation of these technologies have also been provided.

Samenvatting

Dit proefschrift beschrijft het onderzoek en bediscussieert de verschillende aspecten in de Public Health Genomics van infectieziekten voor *Chlamydia trachomatis* en het humaan papillomavirus (HPV). Met ruim 100 miljoen nieuwe infecties per jaar, is *C. trachomatis* de meest voorkomende seksueel overdraagbare bacteriële infectie. Ook HPV infecties komen veel voor in menselijke populaties, met globale prevalentie schattingen die voor vrouwen oplopen van 2% tot 44%. Een HPV infectie met hoog-risico types induceert neoplastische transformatie van geïnfecteerde cellen. Baarmoederhalskanker is een van de meest voorkomende kwaadaardige ziekten bij vrouwen en beslaat bijna 10% van alle kankers bij vrouwen. In dit proefschrift worden studies op genomische gastheer factoren in *C. trachomatis* en HPV infecties gepresenteerd, en wordt deze kennis 'vertaald' naar empirische kennis. Er zijn voortdurende inspanningen om gastheer genoom bevindingen effectief te 'vertalen' naar diagnostiek, preventie en therapie, met het oog op de verbetering van de klinische behandeling van patiënten en de volksgezondheid. Bovendien wordt de bijdrage van gender als een sociaal-culturele factor en health literacy in relatie tot public health genomics van infectieziekten onderzocht en bediscussieerd. Gender is een belangrijke factor die gezondheidsuitkomsten beïnvloedt, maar toch is op dit moment mainstreaming van gender binnen de gezondheidszorg en het medisch onderwijs nog onvoldoende. Ook blijkt uit onderzoek dat er grote tekortkomingen bestaan in de kennis en het begrip van genoom gebaseerde informatie bij gynaecologen en andere specialisten.

Hieronder wordt de inhoud van de drie delen en hun respectievelijke hoofdstukken samengevat.

Deel I van het proefschrift bestaat uit onderzoek naar gastheer genoom (immunogenetische) varianten in infecties met *C. trachomatis* en HPV. In **Hoofdstuk 1** hebben we de rol van functionele polymorfismen in NOD1 en NOD2 receptoren onderzocht. We hebben associaties onderzocht van het *NOD1* +32656 T> GG insertie-deletie polymorfisme met zowel susceptibiliteit voor en ernst van *C. trachomatis* infectie bij vrouwen. Onze resultaten tonen aan een significante vermindering van de houding van de *NOD1* +32656 GG insertie allel in *C. trachomatis*-positieve vrouwen in vergelijking met de *C. trachomatis*-negatieve vrouwen, die wijst op een mogelijk beschermend effect tegen *C. trachomatis* infecties. Aanwezigheid van *C. trachomatis* IgG antilichamen was waargenomen in ernstige onvruchtbaarheid (TFI), die de verwachte relatie tussen vroegere *C. trachomatis* infecties en ontwikkeling van tubapathologie bevestigd. Bovendien, als we *C. trachomatis* positieve vrouwen zonder symptomen met *C. trachomatis* positieve vrouwen met symptomen, en met *C. trachomatis* positieve vrouwen

met TFI vergelijken, zien we een toenemende, statistisch significante trend in het dragerschap van het *NOD1* GG allel [p 0,0003].

De potentiële impact van polymorfismen in drie genen (*VDR* (rs1544410 G> A, rs2228570 C> T), *CYP27B1* (rs10877012 G> T) en *CYP2R1* (rs10741657 G> A)) op de gevoeligheid voor *C. trachomatis* infecties werd vastgesteld in **Hoofdstuk 2**. Het is aangetoond dat genetische variaties binnen de vitamine D metabole pathway de reacties op infecties beïnvloeden. In ons cohort van Nederlandse Kaukasische vrouwen observeerden we echter geen statistisch significant verschil tussen de genotype distributies van die vier polymorfismen. Daarom nemen we aan dat VDR, CYP27B1 en CYP2R1 geen rol spelen in gevoeligheid voor *Chlamydia* infecties, zoals bij een aantal andere infecties werd aangetoond. Niettemin vertonen de genen binnen vitamine D biologische pathway een pleiotrope rol in het immuunsysteem, dus de rol van vitamine D is nog niet te verwerpen voor het gehele klinische verloop van *Chlamydia* infecties en moet verder worden onderzocht.

Hoofdstuk 3 geeft een uitgebreid overzicht van alle bestudeerde immunogenetische polymorfismen die relevant zijn voor HPV-infecties en baarmoederhalskanker. We geven een overzicht van genen die coderen voor cytokines, chemokines, immuunreceptoren en genen die betrokken zijn bij immuunrespons en -regulatie.

Deel II van dit proefschrift richt zich op de start en de afwerking stap van de 'vertaling' van de kennis 'van bench to bedside'. **Hoofdstuk 4** gaat in op de rol van biobanken in het onderzoek van genomics van infectieziekten en de mogelijkheden voor het gebruik van deze kennis voor doeltreffende klinische opname en toepassing. In dit hoofdstuk worden ook prominente voorbeelden gegeven van succesvol gebruik van biobanken op het gebied van gastheer genomics van infectieziekten (namelijk human immunodeficiency virus (HIV), HPV en *C. trachomatis*). Ook worden de belangrijkste kwesties benoemd met betrekking tot de bescherming van persoonsgegevens en het behoud van elektronische medische dossiers in het kader van genomisch onderzoek bij besmettelijke ziekten. Tenslotte worden aanbevelingen voor het beheer van infectieziekte biobanken en reglementaire procedures voor de monsters en data gebruik verstrekt.

In **Hoofdstuk 5** beoordeelden we het translationele potentieel van de "state of the art" genomische en genetische bevindingen voor HIV, *C. trachomatis* en HPV in toepassingen in de volksgezondheid, vooral in diagnose, behandeling en preventie van complicaties van deze infecties. Voor zover ons bekend is dit het eerste review over het translationele potentieel van deze bevindingen voor HIV, *C. trachomatis* en HPV naar toepassingen in de volksgezondheid en in de diagnostiek, behandeling en preventie van late complicaties van deze infectieziekten. We vonden schaarse voorbeelden van de huidige toepassing van genomische en genetische bevindingen, farmacogenomica, en we vonden voorbeelden van genomische informatie met een belofte van 'vertaling' in de nabije toekomst.

Deel III benadrukt twee verwaarloosde gebieden in het onderzoek en de klinische behandeling van de infectieziekten. Onderzoek heeft aangetoond dat gender als sociaal-culturele factor het HPV risico en risico-gedrag, de toegang tot gezondheidszorg en screening rato kan helpen voorspellen, evenals de gevolgen voor de gezondheid, zoals voorgelegd in **Hoofdstuk 6**. De studie legt uit hoe het geslacht is niet alleen een voorspeller van gezondheidsuitkomsten — het is een bepalende factor in de aanvaarding van en de naleving van de klinische technologieën, zoals de HPV DNA test en HPV-vaccin. **Hoofdstuk 7** beschrijft de relevantie van health literacy in gastheer genomische toepassingen. Het hoofdstuk presenteert onderwerpen die relevant zijn voor de acceptatie en implementatie van genoom gebaseerde gezondheidstechnologieën, zoals gastheer trait diagnostische hulpmiddelen in een klinische setting. Om de genoom gebaseerde diagnostische instrumenten met succes te implementeren in de klinische praktijk, is een goed begrip van de genoom gebaseerde informatie vereist van gezondheidswerkers. Onderzoek toont consequent de tekortkomingen in de kennis van gynaecologen en andere specialisten en hun begrip van het genoom gebaseerde informatie aan. Host genomische onderzoek zal in de nabije toekomst het klinische beleid veranderen. Dus is het van cruciaal belang dat zowel professionals in de gezondheidszorg als patiënten en gebruikers in staat zijn om deze informatie behoorlijk te interpreteren. In het hoofdstuk hebben we het onderzoek van onze groep naar de kennis en houding van gynaecologen en artsen ten aanzien van de invoering van genoom gebaseerde technologieën uitgelegd. Daarnaast worden de inspanningen in het betrekken van zorgverleners en patiëntenorganisaties in de uitvoering van deze technologieën weergegeven.

Valorisation

On host genomics, public health genomics and why it matters in infections

Any one person will contract many infectious diseases during their lifetime. Infections have been present throughout humankind's entire evolutionary past, they have shaped our civilisations and changed our cultures, marking their doom and opening new chapters of history. On the other hand, the microbiome in our bodies provides us with an additional line of defence, among other benefits. Next to guns and steel, germs are the ones we largely owe the gratitude for making us what we are today, as Jared Diamond notably depicted it in his most famous piece of work[1]. These microscopic organisms have shaped more than our cultural environment – they successfully (but inadvertently) re-designed our genomes. What came out of tragic events such as the countless epidemics and pandemics of the past were generations that were more resistant to those diseases and are in majority of cases able to fight off the infection. We are the descendants of those who carried slight differences in their genes, the kind of differences that enabled their carriers' immune system to eliminate the potentially deadly infections. These differences, or variants, start off as mutations in the genome. If more than one per cent of the population differs in a certain variant, we refer to it as a single nucleotide polymorphism (SNP). A population's genetic pool of variants enables natural selection to favor those variants that can provide its carrier with a selective advantage under certain environmental conditions (such as exposure to a sexually transmitted infection). If we imagine a gene to be analogous to a sentence in a book[2], the letters in that sentence constitute words, and it is the words (and their assortment) that provide a meaning to the sentence, if arranged in the right order. Changing one or a few letters in a meaningful sentence, like in the case of typos, does not necessarily strip the meaning from a sentence – a reader can often make a good guess what letter should be there instead of the typo. But sometimes the typo can occur in a word that is crucial in giving that sentence meaning and coherency, and the sentence becomes nonsensical. A similar thing happens with mutations in a gene. Some mutations or SNPs hardly affect the "meaning" of a gene, some have huge consequences, and some change the meaning, or "interpretation", under

[1] Diamond, Jared M. *Guns, Germs, and Steel: The Fate of Human Societies.* New York: Norton, 1997.

[2] If we were to broaden this analogy, the book would ideally represent (haploid) human genome, whereas the chapters of the book would correspond to individual chromosomes. All sentences in books, however, are usually meaningful (if you were lucky to stumble upon a good book, that is), and this is where the analogy works to a lesser extent, since chromosomes also have a lot of seemingly "rubbish" "text". I lend my inspiration for these wonderful, highly educational metaphors from Matt Ridley's *Genome: The autobiography of a species in 23 chapters* (New York: Harper Collins, 1999).

certain conditions only. The way a sentence depends on a broader context, the gene and its "translated meaning" depend on the environment – other genes and their products, the cell, the tissue, the entire organism, or the niche that organism dwells in.

There are, however, two sides to the coin called genetic variation. On one hand, mutations in our DNA may lead to numerous and sometimes severely debilitating diseases. For instance, we know of many congenital immune deficiencies caused by these mutations, which may result in the inability of the organism to defend itself against various infections, often in a life-threatening fashion. But more often is the susceptibility caused by different gene variants[3] somewhat subtler, and we can observe this in our everyday lives. Some people we know are ill during the cold season more frequently than others, whereas those others may be more prone to bacterial throat infections for instance.

The field of host genomics has been rapidly mounting evidence on the involvement of different gene variants and their combined effects in infectious diseases, and among them sexually transmitted infections, which are the main focus of this thesis. Our understanding of how cells recognise the presence of particular pathogens and set off a series of immune responses in order to eliminate the threat is becoming greater thanks to the advances in research technologies and unrelenting efforts of researchers. The field of Public Health Genomics offers us with means to prevent and combat diseases more effectively by revolutionising the delivery of health care, as it recognises that at the core of differences in health between individuals and groups lie unique combinations of genetic and environmental influences, and that disease prevention can best be achieved by targeting these influences in a manner tailored to that individual or group. Current health care delivery approach of treating patients and targeting people can best be described as "one size fits all", in which all people suffering from the same illness (or are at risk of being afflicted) are handled in the same way, with the same dosages of same pharmaceuticals or through identical prevention efforts. But different people react differently to the same dose of a particular medication, and not everyone requires the same dose, or in some cases the same medication. Similarly, not everyone's immune system is identical, therefore different persons may have different experiences in infections, such as *Chlamydia trachomatis* for instance. Some individuals rid the infection quite quickly and often without even knowing it – their immunity is well-equipped for eradicating that type of bacteria. Others, however, may suffer longer and harder from the infection, and the pathogen can spread up their reproductive organs up to the fallopian tubes, causing severe defensive reactions by the immunity, where one's own body gets damaged in a desperate attempt to fight off the threat. And then there are those who are not able to

[3] Susceptibility to infectious diseases, as well as their severity, typically depends not solely on genetic – and other biological – factors, but the environmental (including socio-cultural) conditions, as well as virulence factors that come from the variation in the pathogen itself. Precise role of these other elements in susceptibility to and severity of infections is beyond the scope of this chapter.

eliminate this bacteria fast, but their immune attacks also wreak less havoc – due to their particular gene variants – These individuals, however, usually manage to rid their disease too, it simply takes them longer time.

Therefore, the one-size-fits-all paradigm in health care does not stand in the face of evidence any longer. Public Health Genomics is laying the groundwork for future medical advances which would be based on a more comprehensive knowledge on the role of both genes and the environment and their interplay. This thesis provides additional insights into the role of different variants in genes which code for receptor molecules capable of sensing the presence of *C. trachomatis* and triggering immune reactions to it. Our research shows that particular SNPs in these genes can be associated with differences in how the body clears this infection. We also provide hypotheses regarding the possible role of these receptors in developing damage to the tubae in women who have been infected with *C. trachomatis* at a certain point. We are still compiling the knowledge necessary for full understanding, hence research of the involvement of these factors is highly relevant, it needs a continuous effort of researchers and sufficient means allocated to this field of research. Given the multifaceted impact and relevance of this knowledge and potentials for its implementation into the clinics and health care in general, empirical knowledge on host genomic factors is of interest to other various target groups: health care professionals and providers, health care consumers, medical education community, policy makers and the governmental legislative branch officials, and insurance providers among others. Media also bears responsibility in saliently informing the public on the use of host genomic data.

On diagnostics of Chlamydia trachomatis-related subfertility

Subfertility is a considerable global health issue and poses a tremendous burden not only on individuals suffering from it, but also on health care systems. Subfertile couples experience difficulties in conceiving. According to the estimates, around 15% of the couples trying to conceive are affected by subfertility. Infection with *C. trachomatis* can sometimes be the cause of a number of problems with the tubae, including the difficulty to conceive. Aside from tubal damage, ectopic pregnancy, untreated prolonged infection might lead to full-fledged infertility. Due to the absence of symptoms, women are often unaware of the infection, hence they do not seek treatment. It may also be that making a diagnosis or getting treated is not possible, in cases where a woman lacks access to health care services.

The costs associated with subfertility run high, which is only in part caused by the need for treating diseases caused by *C. trachomatis* infection. Current protocols for diagnosing tubal pathology require that a woman who has difficulties conceiving and tests positive for *C. trachomatis*[4] needs to undergo laparoscopy and other invasive procedures. Not only is this procedure very costly – it is invasive and stressful for the woman, and it carries a risk of surgical complications. Finally, it is estimated that over 40% of women who have had laparoscopy performed do not suffer from tubal pathology. Therefore, a diagnostic product that would enable us to better target those women who are more likely to develop tubal pathology after having had *C. trachomatis* would spare these women the psychological burden and inconvenience brought about by a laparoscopy procedure, and would also greatly reduce the burden on health care, both in terms of financial and human resources. Also, up to 20% of women with subfertility issues who do not test positive for past *C. trachomatis* infection – as shown by serology tests – do at some stage develop tubal pathology. However, due to the current protocols will be undetected and will not receive the needed intervention in a timely manner.

Several studies presented in this thesis have investigated how specific gene variants in receptors that signal the presence of *C. trachomatis* infection can greatly affect whether a woman will build a successful immune defence in response and whether she is likely to suffer tubal damage as a consequence of its own defence mechanisms (the consequence of which may be subfertility). We already know from other studies that the risk of these afflictions adds up when a woman with a positive serology test for *C. trachomatis* carries two or more of particular variants in these genes. These women are two and a half times more likely to have tubal pathology than those who have one or none of these variants. So there is a tremendous innovative potential in developing a diagnostic tool that would help health professionals distinguish between these two groups of women.

Therefore, our goal is to develop a diagnostic assay that tests for the presence of these variants and helps clinicians assess if a woman should indeed undergo laparoscopy. The end result would be improved clinical triage and diagnosis of women with subfertility issues. This assay would bring innovation to infectious disease diagnostics, as this will be the first ever diagnostic application in the area of infections and infection-associated complications based on genetic variants. The assay is under development together with all the key stakeholders involved.

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[4] Serology testing cannot differentiate between an infection in the past and an ongoing one with complete precision. In addition to that, there is a considerable number of false negatives (when the test gives a negative result even though the infection occurred). Nevertheless, serology tests provide an indication for diagnosis, albeit not fully accurate. Tests that detect *C. trachomatis* DNA are increasingly used, but they do not tell us anything on whether a person had been infected prior to several weeks in the past.

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A straight line, gently inclined at a six-ish degree angle, giving a slow but steady climb up to the end point. No major meandering or surprising detours of any kind. That is how I envisioned my PhD period, back then at the very start. I suppose I can laugh about my naïve presumptions now. For a phrase in singular, 'learning curve' seems to be teeming with too many curves. But I am unexpectedly glad things got bumpy every now and then. It was a growth process, and I don't feel like it ends here.

All of this would not have been possible without the help of my supervising team – Prof. Dr. Servaas A. Morré, Dr. Sander Ouburg, and last but not least, Dr. Ineke Klinge. I would first like to give my gratitude to Servaas, my main supervisor, for his guidance and support. One must appreciate when a supervisor is available for calls even at odd hours of the night, replies to e-mails and text messages in the middle of meetings, and stays up until dawn giving feedback to your manuscript drafts. Sander, I feel that I have greatly benefitted from working together with you. Absorbing knowledge from you feels easy, because you make it easy. (Even if that entails applying the V.O.C. method). Ineke, thank you for kindly providing your expertise and advices. It was especially important during the initial periods of my trajectory, shall we say my PhD baby steps? I still wonder from time to time: whatever happended to Madovny?!

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Curriculum Vitae

Ivan Branković was born on February 8th 1980, in Belgrade, Serbia (former Yugoslavia). In 1999, he finished his secondary education (gymnasiym, section mathematics and natural sciences) in Obrenovac. The following year, he began his diploma studies in general biology (equivalent to BSc + MSc degree) at the University of Belgrade, final year orientation Microbiology and Immunobiology, for which he obtained a degree in 2007. Upon receiving the MTEC scholarship funded by the Dutch government, he went on to do Master of Science studies in Public Health at Maastricht University (field: Health Education & Promotion). He obtained the degree in August 2008. For his final thesis, he conducted a study of the effect of the cleanliness of the living environment on Dutch and Serbian students' perceived susceptibility of contracting a sexually transmitted infection.

In 2009, he came back to Maastricht University to work as a researcher. In the period 2009-2011 he was conducting research for the EUGiM project, researching sex and gender factors of infectious and autoimmune diseases for the purpose of developing an educational module in gender medicine. Since June 2011, he started working on his PhD thesis under supervision of Prof. Dr. Servaas A. Morré at the Institute for Public Health Genomics. In the thesis, he focuses on public health genomics of *Chlamydia trachomatis* and human papillomavirus (HPV) and their association with host susceptibility to and severity of related sequelae, tubal factor infertility and cervical cancer, respectively. The thesis also covers the state of the art in translational research, namely opportunities for implementation of the findings on host genomic factors into the clinical setting, for the purpose of improving clinical management of patients with subfertility issues, as well as prevention and therapy. His practical laboratory work was based at VUmc Laboratory of Immunogenetics, where he is still involved in ongoing research of immunogenetic factors of *C. trachomatis* and HPV. He hopes to become an established researcher in the field of host genomic research of sexually transmitted infections.

List of publications

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Branković I*, Lanjouw E*, Ouburg S, Pleijster J, Spaargaren J, van Kranen HJ, Hoebe CJPA, & Morré SA. **Functional polymorphisms in vitamin D metabolism genes are not associated with susceptibility to *Chlamydia trachomatis* infection**. [submission in progress].

Mooij M.*, Branković I.*, Morré S. A., & Ouburg S. **Profiling HPV infection and cervical cancer: a comprehensive overview of investigated immunogenetic polymorphisms.** [submission in progress].